SYNTHESIS OF BELACTOSIN A ANALOGS

by

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ABSTRACT

SYNTHESIS OF BELACTOSIN A ANALOGS

There is continuing interest in new research on cancer as well as new cancer drugs. Belactosin A, discovered in 1999, is a naturally occurring 26S proteasome inhibitor with anti-tumor activity. The 26S proteasome is a novel anti-tumor target with only two inhibitors on the market. The structure of belactosin A contains a cyclopropyl amine linked to a β-lactone. While several syntheses have been reported, only a few analogs have been prepared and analogs are needed since belactosin A is toxic.

Approaches to the synthesis of alanine cyclopropyl analogs as well as the synthesis of a simple beta-lactone are reported here. The synthetic route included a nitrocyclopropanation as well as a transfer hydrogenation to afford the core cyclopropyl amine of the analogs. Issues regarding the type of protecting group used on the "left side" of the analogs as well as attempts toward the removal of the ketone have been investigated. Attempts to couple the monoamine to the beta-lactone as well as the stability of the beta-lactone have been studied as well.

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CHAPTER ONE: INTRODUCTION

Cancer Drug Design

All living cells go through the same process of growth, replication, and more growth in preparation for cell division and finally mitosis. This is followed by cytokinesis as shown in Figure 1.

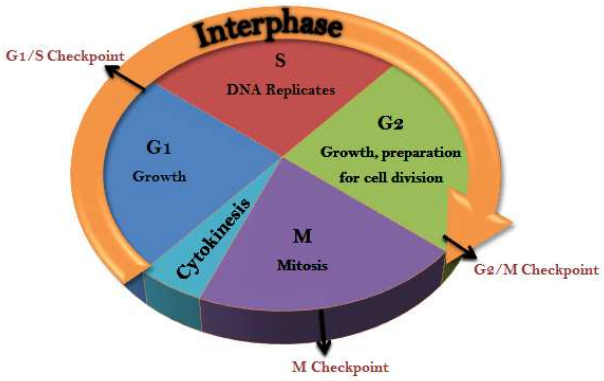


Figure 1: This image shows the cycle of a cell where G represents the growth phases and S represents the synthesis phase.

Cancer cells are not exempt from this cycle; however with cancer cells, this cycle perpetuates and the cells divide rapidly unless it is caught and dealt with medicinally. Two checkpoints within the cell cycle that use extracellular signals are the G_1/S checkpoint and the G_2/M checkpoint. At the G_1/S checkpoint, the cell can transition either to start DNA replication or to go into a rest phase known as G_0. At the G_2/M

checkpoint, the cell transitions to start the mitosis process. Both of these checkpoints utilize extracellular signals to move the cell cycle forward. Cancer cells abandon these controls (signals) and tend to remain in cycle.[1] Since cell cycle exit can facilitate maturation and terminal differentiation, these processes are subverted as well.[1] In the design of oncological drugs, the goal is to find targets or areas in the cell cycle to attack or cause inhibition that will ultimately causes apoptosis or programmed cell death.

There are many different types of cancer drugs on the market, which act by several different mechanisms. Almost all cancer drugs target general cell replication. Traditionally, anticancer drugs target DNA synthesis and are cytotoxic drugs. Cytotoxic drugs work by targeting cells that proliferate frequently, but in so doing can affect normal cells that are constantly produced, such as immune cells. These drugs also cause a wide range of side effects such as nausea, hair loss, and immune suppression. A few of the current drugs on the market for this type of therapy are 5-fluorouracil (5-FU), doxorubicin, and taxol, as seen in Figure 2.

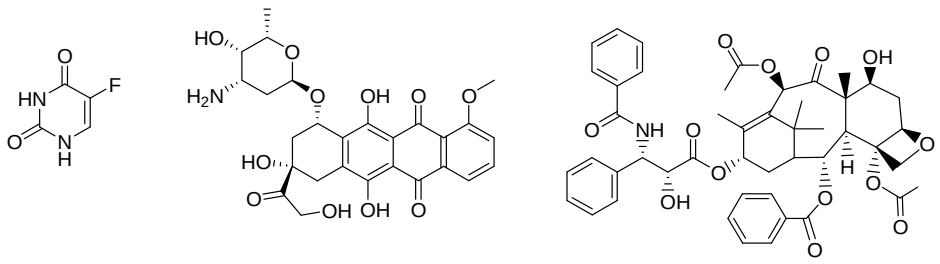


Figure 2: Structures of 5-fluorouracil, doxorubicin and taxol from left to right.

All of these act on either DNA or the cell cycle. 5-FU inhibits an enzyme, thymidylate synthase that is involved in DNA synthesis.[2] Doxorubicin intercalates between DNA

base pairs, disrupting the helix. Taxol binds to microtubules, which forms the spindle apparatus, interfering with mitosis.[2]

Newer drugs that are now on the market, known as targeted therapies, work by targeting proteins that are different in cancer cells than normal healthy cells. Many of the targeted therapy drugs target the tyrosine kinases as well as cyclin-dependent kinases. Kinases are enzymes that play a role in the phosphorylation process, which helps transmit signals from the cell. Tyrosine kinases and the cyclin-dependent kinases are over-expressed and/or mutated in cancer cells, which make them good targets for cancer therapy. Even newer targets for therapy in cancer treatment are proteasomes, specifically the 26S proteasome. Proteasomes are complexes of proteins that help in the degradation of proteins that are not needed or are damaged.[3]

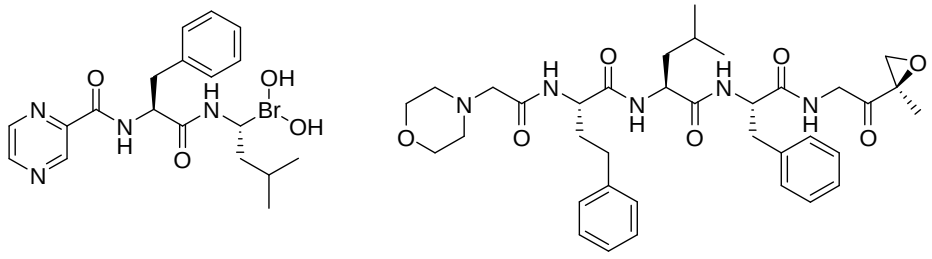


Figure 3: Structures of bortezomib and carfilzomib respectively.

Bortezomib and carfilzomib, pictured in Figure 3 above, are both proteasome inhibitors approved for the treatment of multiple myeloma.[3,4,5] Bortezomib and carfilzomib work by inhibiting the 20S core particle of the 26S proteasome and thus effectively stop the degradation of certain proteins that cause cell growth and mitosis.

Carfilzomib was approved by the FDA in 2012 and other proteasome inhibitors are currently in clinical trials, with medicinal chemists around the world showing interest in this new molecular target.[5]

The 26S proteasome, shown in Figure 4, is made up of a 20S core together with one or two 19S regulatory particles.

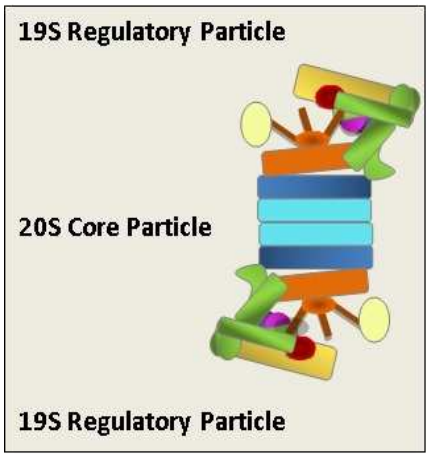


Figure 4: The human 26S proteasome

The 20S core is formed of four stacked rings of seven highly homologous subunits arranged as a $\alpha_7\beta_7\beta_7\alpha_7$ barrel.[6] The active site for the inhibition of this proteasome is within the barrel at the N-terminals of β1, β2, and β5. The 19S regulatory particles are described as being complexes with an ATPase unit and a non-ATPase unit. They are composed of at least 18 subunits, which serve to recognize, unfold, and translocate the substrate proteins into the proteolytic core.[6]

Belactosin A and Analogs

Discovery and first synthesis.

While working on the microbial screening program of *Streptomyces* bacteria, Asai and coworkers discovered a novel metabolite known as belactosin A.[7] Asai *et al.* discovered that this metabolite had physicochemical properties as well as biological activity specifically inhibiting cell cycle progression of human tumor cells at the G2/M phase.[7] Belactosin A, pictured in Figure 5, was discovered in 1999 and just four years later was synthesized in the lab of Alan Armstrong at the Imperial College of London.

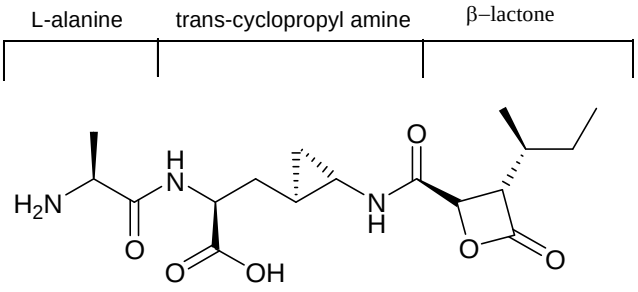


Figure 5: Structural features of belactosin A.

The structure of belactosin A consists of three main components: L-alanine coupled to a *trans*-cyclopropyl amine, which forms an amide with a β-lactone entity. The structure activity studies on belactosin A show that the active portion of the molecule, the β-lactone, is attacked in the 20S core of the proteasome effectively opening the lactone ring and inhibiting the proteasome.

Armstrong was the first to synthesize belactosin A by coupling a β-lactone carboxylic acid with *N*-ala-aminocyclopropyl alanine as shown in Figure 6.[8]

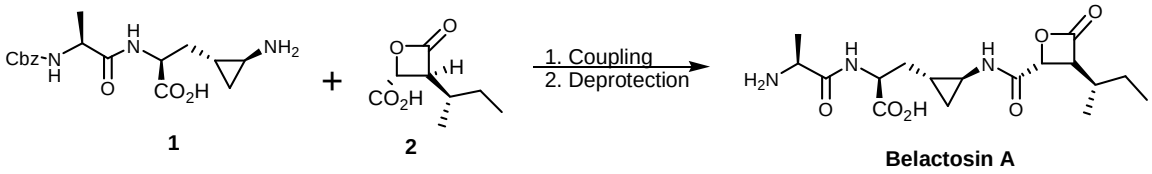
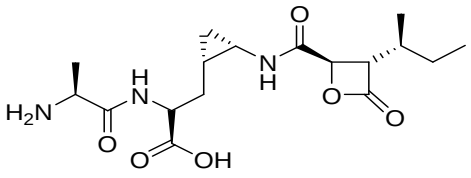


Figure 6: Armstrong's synthesis of belactosin A.

The total synthesis took 13 total steps, resulting in a 7.2% overall yield. Armstrong first synthesized a protected version of intermediate **1** to couple to a protected L-alanine, then synthesized the β-lactone *via* a stereoselective chlorination, and followed this by cyclization. Once these two pieces were synthesized, Armstrong coupled the two products, followed by deprotection of the carbamate to form belactosin A.

Analogs.

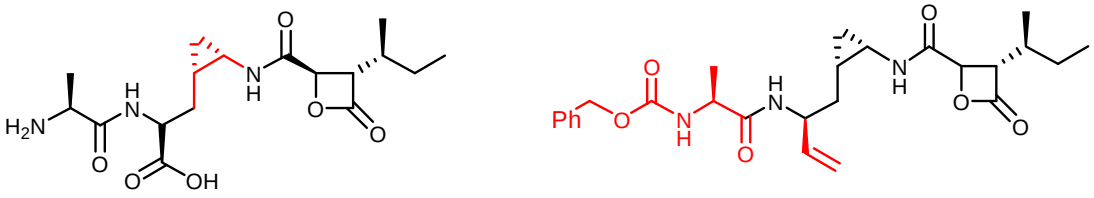
Since the first synthesis of belactosin A, other groups have started working on synthesizing analogs to create compounds that are more potent and less toxic. The most recent group to synthesize analogs of belactosin A is Kawamura *et al.*[9] Kawamura *et al.* worked on analogs incorporating the *cis*-cyclopropane stereoisomer **3**, as shown below in Figure 7, and synthesized 27 different analogs of belactosin.



3

Figure 7: Structure of the *cis*-isomer of belactosin A.

The areas the group changed are highlighted in red below in Figure 8. Changes included the *cis* stereoisomer of the cyclopropyl **3**, and substitutions for the carboxylic acid and acylation of the terminal amine **4**.



3 **4**

Figure 8: Analogs of belactosin synthesized by Kawamura *et. al.* with changes to the structure highlighted in red.[9]

The most potent inhibitor that Kawamura *et al.* synthesized was what they called compound **3e** shown below binding to a threonine residue within the 20S proteasome. Important features to note are the lipophilic phenethyl replacement for the acid and the carbamate analog of the alanine.

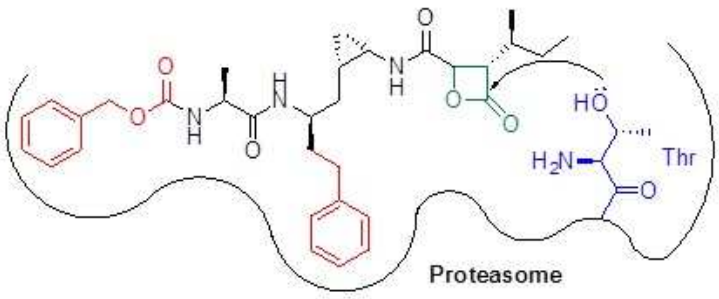
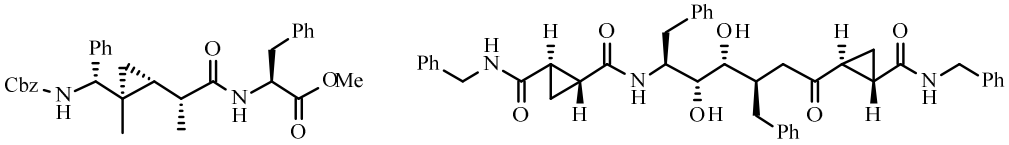


Figure 9: Compound 3e binding to 20S proteasome[7]

The research done by Kawamura *et al.* allows for optimization for this interesting class of proteasome inhibitors.[9]

Peptidomimetics

Peptidomimetics are compounds whose essential elements (pharmacophore) mimic a natural peptide or protein in 3D space which retain the ability to interact with the biological target and produce the same biological effect.[10] Peptidomimetics are useful in drug development because these compounds can be used to activate or inactivate a protein, proteasome, or other biological target. Peptidomimetics come in many different types, some that resemble a peptide and others that have no similarities to a peptide. One type of peptidomimetic that has been proven to have anti-tumor, antiviral, and antidepressant activity are the cyclopropyl peptidomimetics.[11]



Wipf's cycloprpyl tripeptide isostere
5

Martin's HIV protease inhibitor
6

Figure 10: Structures of cyclopropyl peptidomimetics 5 and 6.

Two examples of cyclopropyl peptidomimetics are Wipf's cyclopropyl dipeptide isosteres **5** and Martin's HIV protease inhibitor **6** as shown in Figure 10. Cyclopropyl peptidomimetics can be used in the synthesis of peptide-like belactosin A analogs since belactosin A has a cyclopropyl amino acid.

Approaches.

There are different approaches to peptidomimetics. The basic approach is the use of either natural or unnatural amino acids in the synthesis of compounds to mimic a peptide, while using a replacement for at least one amide bond. Structure-activity relationships (SAR) can determine which side chains are needed for binding sites on the biological target. For the case of cyclopropyl peptidomimetics, two ways to generate the cyclopropyl core have been investigated in this research group. Both are three step syntheses, shown in Figure 11, that begin with protected amino acids.

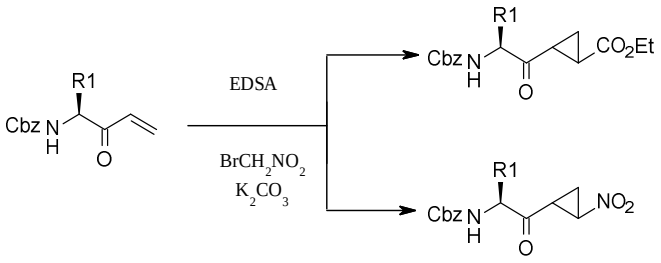


Figure 11: Synthesis of ester and nitro-cyclopropyl peptidomimetics[9]

One uses EDSA [ethyl (dimethyl-λ^4-sulfanylidene)acetate] and the other uses bromonitromethane for the cyclopropanation.[12] Since belactosin A has the cyclopropyl core this method can be used in the synthesis of belactosin A as well as the analogs of belactosin.

Goals of Project

The main goal of the project is to synthesize two analogs of belactosin A. Three objectives need to be complete to finalize the main goal of the project. The first objective is the synthesis of a simple β-lactone. The second goal is the synthesis of belactosin A analogs containing the simple β-lactone, and a hydroxyl substituent. Finally, the third goal is the synthesis of a methyl analog of belactosin with and without the hydroxyl group. Once the synthesis is complete, biological activity assays will be done to see how these changes affect the activity of belactosin A.

Synthesis of simple β-lactone.

The first goal of the project is to synthesize a simple β-lactone. The synthesis will follow the first three steps of the Cammas *et al.* procedure shown in Figure 12.[13]

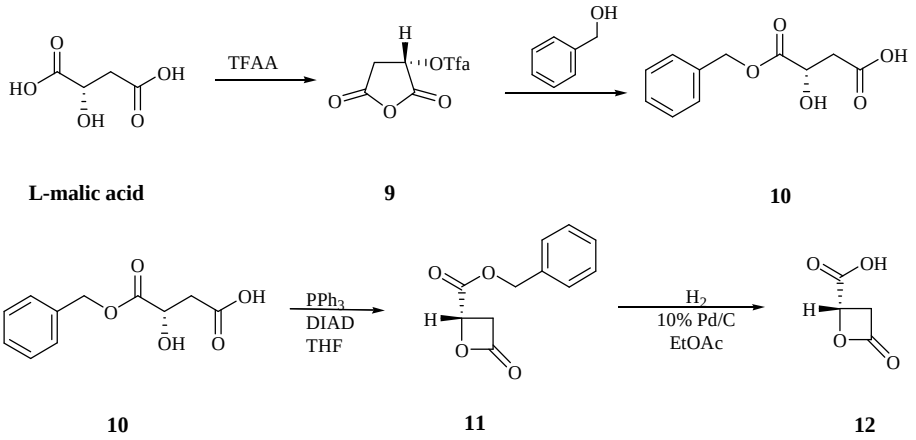


Figure 12: Synthesis of Simple β-Lactone

The Cammas *et al.* procedure is for the synthesis of poly (β-malic acid) derivatives, but it does give the (R)-β-substituted β-lactones with very high enantiomeric excess.[13] These compounds have to be handled with extreme care to prevent them from polymerizing. In this synthesis, L-malic acid is treated with trifluoroacetic anhydride (TFAA) to form a cyclic intermediate, which is then immediately treated with benzyl alcohol to produce **10**. The product **10** with then undergoes a Mitsunobu reaction with triphenyl phosphine and diisopropyl azodicarboxylate (DIAD) in tetrahydrofuran (THF) to close the lactone ring. The last step in the synthesis of the simple β-lactone is the hydrogenation of **11** with hydrogen gas and palladium catalyst to get the malolactonic acid **12**. According to Leboucher-Durand *et al.*, **12** is obtained in a 99% yield using hydrogenation.[14]

Synthesis of analogs.

The next goal in the synthesis of belactosin A analogs is the synthesis of a methyl analog with a hydroxyl component of belactosin using the simple β-lactone (12) as well as one without a hydroxyl component.

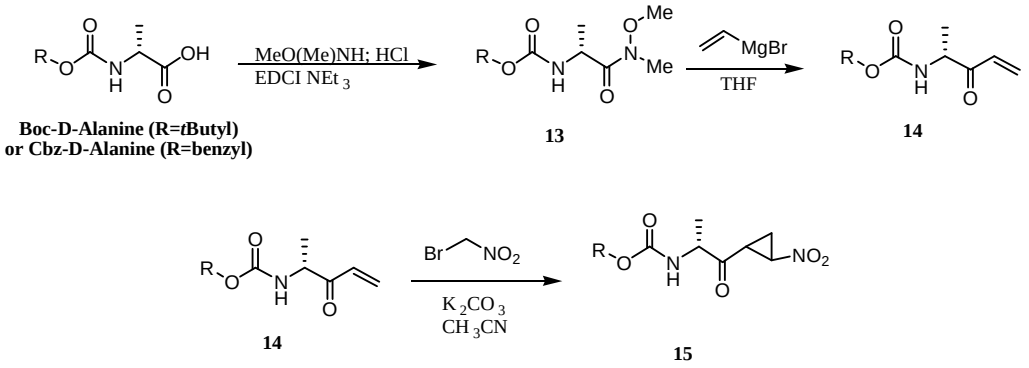
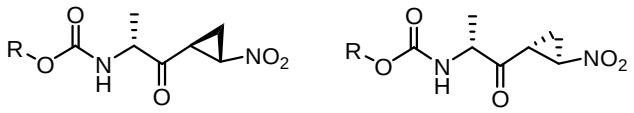


Figure 13: First steps of reaction scheme for analogs

The synthesis of these two analogs will follow the same steps initially as shown in Figure 13. The first step of the synthesis of the methyl analog of belactosin will start with the formation of a Weinreb amide from a (Boc or Cbz) protected D-alanine. The amide will then be converted into an enone using a vinyl Grignard reagent. The next step of the synthesis is the cyclopropanation step using bromonitromethane, potassium carbonate in acetonitrile using the procedure described by Dunlap *et al.*[11] The cyclopropanation step produces *syn* and *anti* diastereomers as shown in Figure 14.



***Anti*-15** ***Syn*-15**

Figure 14: Diastereomers of compound 15

Once the cyclopropyl core is produced, the synthesis diverges for the two analogs. For the analog with the hydroxyl component, the ketone is reduced to an alcohol by sodium borohydride as shown in Figure 15.

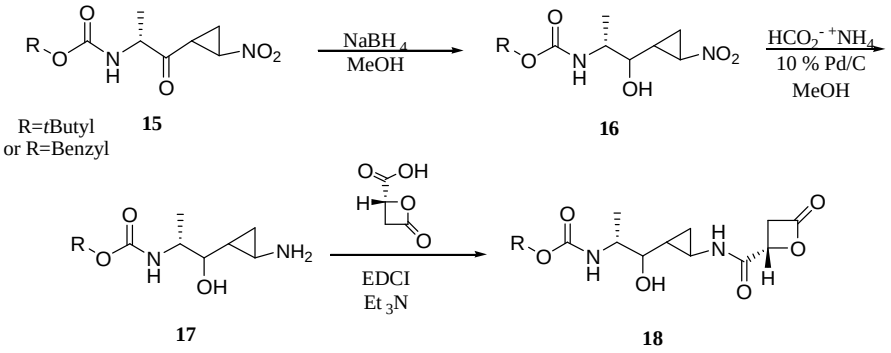


Figure 15: Reaction scheme of hydroxyl analog

Once the ketone is reduced, the next step is to reduce the nitro group to a free amine by transfer hydrogenation using ammonium formate with palladium catalyst. The free amine will then be coupled to the simple β-lactone **12** using EDCI and triethyl amine producing the first analog of belactosin.

The next goal in the synthesis of the analogs is the synthesis of a methyl analog without the hydroxyl component. This synthesis as shown in Figure 16 only changes after the cyclopropanation step.

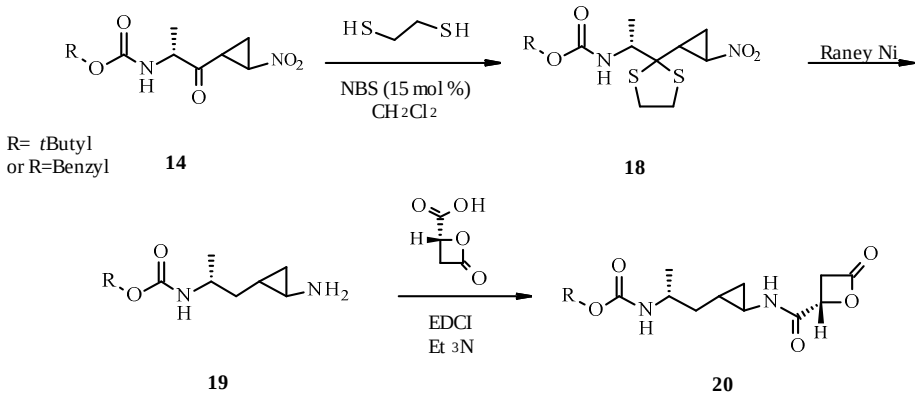


Figure 16: Reaction scheme of analog without hydroxyl component

In this reaction scheme, the ketone is converted into a dithioacetate utilizing N-bromosuccinimide and 1, 2-dithioethane using the procedure described by Kamal.[15] The dithioacetal is then reduced along with the nitro group by Raney nickel. Upon desulfurization, the product will then be coupled to the β-lactone as was done in the first analog synthesis. Once synthesized, these compounds will be tested for biological activity. The biological activities that will be tested are anticancer, anti-bacterial, as well as antiviral.

CHAPTER TWO: METHODS AND MATERIALS

Summary of Syntheses

β-Lactone.

The synthesis of the β-lactone started with commercially available L-malic acid that was converted into the trifluoroacetate of malic acid **9** as shown below in Figure 17. The anhydride was immediately treated with benzyl alcohol to produce **10**, a benzyl protected form of L-malic acid. The benzyl protected L-malic acid underwent a Mitsunobu reaction to form then benzyl protected β-lactone **11**. The benzyl-protected lactone was then converted to the respective carboxylic acid **12** by hydrogenation.

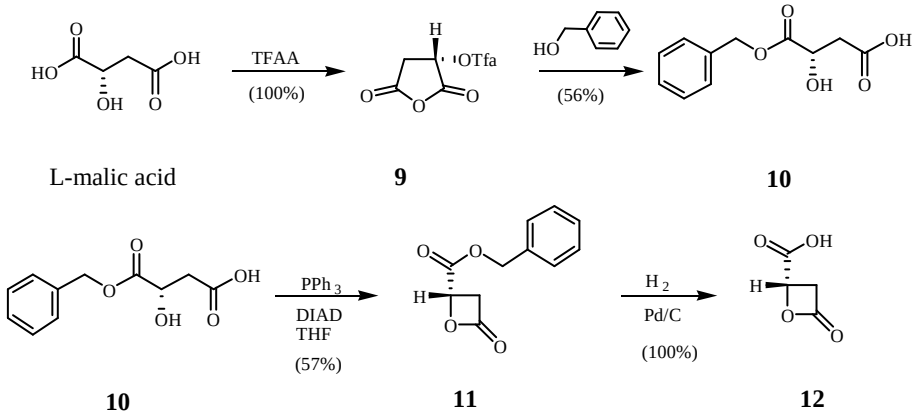


Figure 17: Synthesis of β-lactone

Methyl (hydroxyl) analog.

As shown below in Figure 18, the synthesis of the methyl analog with hydroxyl component began with a Boc protected D-ala enone **21**. Nitrocyclopropanation of the

enone produced *syn* and *anti* diastereomers of the cyclopropyl nitro ketone **22**. The ketone was reduced to form a mixture of isomers of the cyclopropyl nitro alcohol **23** and this was followed by a transfer hydrogenation that produced the amine **24**. Upon the hydrogenation, the amine was then coupled to the β-lactone **12** to attempt to produce the analog **25**.

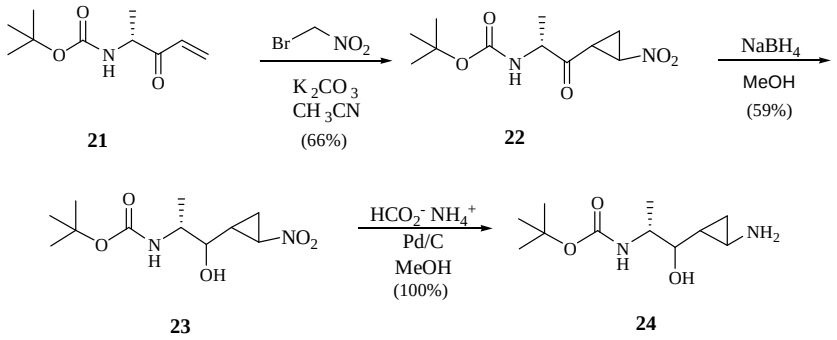


Figure 18: Synthesis of methyl analog with hydroxyl component

Attempts were made to couple the β-lactone **12** to benzyl amine as well as the cyclopropyl amine **24** as shown in Figure 19. While benzyl amine did couple to some molecule within the reaction mixture, amine **24** did not appear to couple to anything.

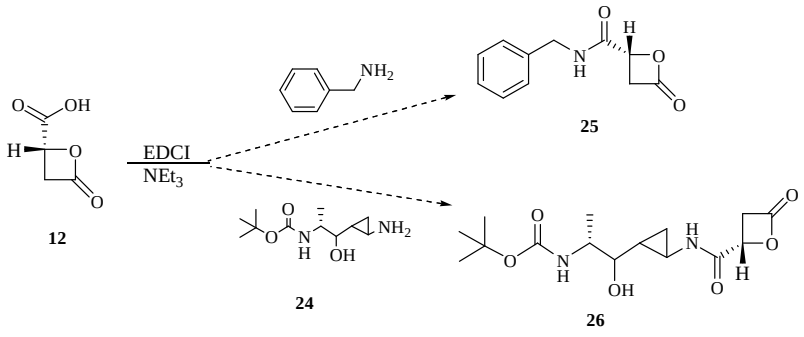


Figure 19: Attempts to couple β-lactone

Attempted Synthesis of Methyl Analog.

The synthesis of the methyl analog without the hydroxyl proved to be difficult. The synthesis, as shown in Figure 20 below, started with N-(Cbz)-D-alanine, which was converted to the Weinreb amide **26**.

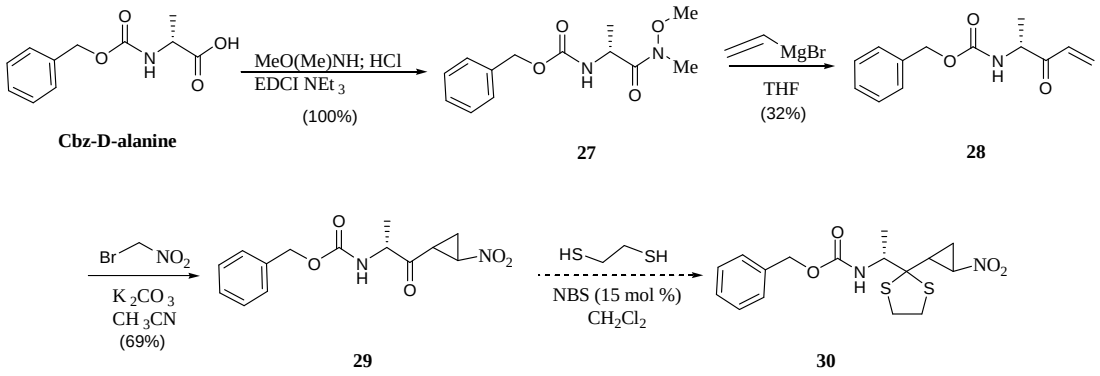


Figure 20: Synthesis of Thioacetate

The Weinreb amide was converted to a terminal enone **27** by addition of vinylmagnesium bromide. The enone then underwent a nitro-cyclopropanation to produce a mixture of *syn* and *anti* diastereomers cyclopropyl nitro ketone **29**. Several attempts to convert the ketone to a thioacetal **30** were carried out, but the product has not been confirmed.

Instruments, Materials, and Reagents

The NMR data were obtained by using a 500 MHz FT-NMR model ECA-500 JEOL (Peabody, MA) purchased with funding provided by the National Science Foundation through the NSF-RUI program (#0321211) and where indicated a 300MHz FT-NMR

model ECA-300 JOEL (Peabody, MA). Chemical shifts are reported in parts per million using tetramethylsilane (TMS) as an internal reference. Splitting patterns are designated by the following: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets) and ddd (doublet of double doublets). High resolution electrospray ionization-mass spectrometry (ESI-MS) was performed at Notre Dame University, Notre Dame, Indiana.

Thin layer chromatograpy (TLC) was performed on glass plates coated with silica gel and UV active backing purchased from Fisher Scientific, Pittsburgh, PA. The TLC plates were analyzed with a short wavelength (254 nm) UV light and subsequently stained with phosphomolybdic acid (reagent grade, Aldrich, Milwaukee, WI) prepared as a 10% solution in ethanol. Column chromatography was performed with silica gel, 60 Å 230-400 mesh ASTM (reagent grade, Fisher Scientific, Pittsburgh, PA) and flash chromatography was performed on an ISCO CombiFlash R_f 200 (Teledyne ISCO, Lincoln, NE).

Methylene chloride, methanol, acetone, acetonitrile, ethyl acetate and hexanes were purchased from Fisher Scientific, Pittsburgh, PA. Chloroform was purchased reagent grade from Acros Organic, New Jersey, USA. Deutero-chloroform ($CDCl_3$) was purchased from Aldrich, Milwaukee, WI and Cambridge Isotope Laboratories, Inc., Andover, MA. Solvent extractions were performed using ethyl acetate or methylene chloride where indicated and washed with 1M HCl, saturated sodium bicarbonate, and brine (reagent grade, Fisher Scientific, Pittsburgh, PA). The organic layer was dried with magnesium sulfate (Fisher Scientific, Pittsburgh, PA) and filtered. Evaporation of solvents was achieved using a Buchi rotary evaporator (Model RII, Buchi, Switzerland).

Triethylamine (Et_3N) was obtained from Fisher Scientific, Pittsburgh, PA. Anhydrous reagent grade vinylmagnesium bromide and tetrahydrofuran (THF) were purchased from Aldrich, Milwaukee, WI. Other reagents including L-malic acid, trifluoroacetic anhydride (TFAA), benzyl alcohol, triphenylphosphine (PPh_3), diisopropyl azodicarboxylate (DIAD), N,O-dimethyl hydroxylamine hydrochloride, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), bromonitromethane, potassium carbonate, sodium borohydride, 10% palladium on carbon, dithioethane, N-bromosuccimide (NBS), and ammonium formate were obtained from Aldrich, Milwaukee, WI.

Synthetic Methods

Synthetic methods for synthesis of β-lactone.

(S)-2,5-dioxo-tetrahydrofuran-3-yl 2,2,2-trifluoroacetate (9). L-malic acid (1 g, 7.82 mmol) was placed in a 25 mL round bottom flask and cooled to 0°C. Trifluoroacetic anhydride (TFAA) (2.2 mL, 15.62 mmol) was added slowly and the mixture was allowed to stir at 0°C for one hour and at room temperature for two hours. Excess TFAA was removed by vacuum distillation to afford 1.60 g product (100%). The product was immediately used in the next step of the synthesis without further purification.

(S)-4-(benzyloxy)-3-hydroxy-4-oxobutanoic acid (10). The trifluoroacetate of malic acid anhydride (**9)** (1.6 g, 7.82 mmol) was dissolved in benzyl alcohol (0.8 mL, 7.82 mmol). The reaction was stirred at room temperature overnight. The oil was then

dissolved in 50 mL of ethyl acetate and was extracted with three portions of sodium bicarbonate. The aqueous layers were then combined and acidified to a pH of 2 with 1 M hydrochloric acid. The aqueous layer was then extracted three times with ethyl acetate. The combined organic layers were dried with anhydrous magnesium sulfate and the solvent was evaporated. The crude product was chromatographed on 25 x 120 mm silica gel eluting with 80% ethyl acetate in hexane to afford 0.952 g (56%) of the product **10**. ^{1}H-NMR (500 MHz, D_6-acetone): δ 7.37-7.34 (m, 5H, aryl), 5.16 (s, 2H, C$\underline{H_2}$Ph), 4.56-4.55 (m, 1H, C$\underline{H}$OH), 2.80-2.70 (m, 2H, C$\underline{H_2}$COOH); ^{13}C-NMR (125 MHz, D_6-acetone): δ 172.9 ($\underline{C}$OOH), 171.2 (benzyl ester $\underline{C}$=O), 136.3 (4 º aryl $\underline{C}$), 128.6-128.1 (5 aryl $\underline{C}$), 67.7 ($\underline{C}H_2$Ph), 66.4 ($\underline{C}$HOH), 38.2 ($\underline{C}H_2$COOH).

(S)-benzyl 4-oxooxetane-2-carboxylate (11). To a solution of the benzyl ester **10** (0.4.826 g, 2.15 mmol) in 6.85 mL tetrahydrofuran (THF), triphenylphosphine (PPh_3) (0.5639 g, 2.15 mmol) was added and stirred at a temperature of 0°C for ten minutes. A solution of diisopropyl azodicarboxylate (DIAD) (0.4348 g, 2.15 mmol) and 1.33 mL THF was added to the benzyl ester solution and the mixture was stirred for 30 minutes at 0°C. After 30 minutes, the ice bath was removed and the reaction was allowed to stir for 20 hours at room temperature. The solvent was evaporated and crude product was chromatographed on 20 x 120 mm silica gel eluting sequentially with 1:9, 1:8, 1:7, and 1:5 ethyl acetate-hexane to afford 0.2522 g (57%) of the ester-protected β-lactone **11**. IR (ν, cm^{-1}): 1848 cm^{-1} (C=O, lactone), and 1749 cm^{-1} (C=O, ester); ^{1}H-NMR (300 MHz D_6-acetone): δ 7.39-7.36 (m, 5H, aryl), 5.24 (s, 2H, C$\underline{H_2}$Ph), 4.90-4.83 (m, 1H, C$\underline{H}$ lactone), 3.77-3.56 (ddd, 2H, C$\underline{H_2}$ lactone); ^{13}C-NMR (75.6 MHz D_6-acetone): δ 168.2 ($\underline{C}$=O

ester), 166.2 (C=O lactone), 134.7 (4º aryl C), 129.0-128.7 (5 aryl C), 67.9 (CH lactone), 65.4 (CH_2 ester), 43.5 (CH_2 lactone).

(S)-4-oxooxetane-2-carboxylic acid (12). To a solution of the ester **11** (56 mg, 0.272 mmol) in 20 mL of ethyl acetate was added 56 mg of 10% palladium on carbon. The solution was placed under hydrogen gas on a Parr shaker at 36 psi for 19 hours. The solution was filtered over Celite twice and rinsed with ethyl acetate to afford a quantitative yield of the carboxylic acid β-lactone **12**. ^{1}H-NMR (500 MHz, d_6-acetone): δ 4.87 (d, 1H, CH lactone) and 3.83-3.52 (ddd, 2H, CH_2 lactone); ^{13}C-NMR (125 MHz, D_6-acetone): δ 169.4 (C=O lactone), 166.8 (COOH), 65.3 (CH_2 lactone), 43.0 (CH lactone) identical to literature.[14]

Synthetic methods of Boc-Ala analog.

***tert*-Butyl (R)-1-(2-nitrocyclopropyl)-1-oxopropan-2-ylcarbamate 22.** To a solution of Boc-ala-enone **21** (0.544 g, 2.78 mmol) in 12 mL of methylene chloride on ice was added crushed potassium carbonate (0.384 g, 2.78 mmol) followed by one equivalent of bromonitromethane (194 μL, 2.78 mmol). After 30 minutes, ice bath was removed and two more equivalents of bromonitromethane were added at two hour intervals. After a total of six hours, the reaction solution was diluted with ethyl acetate, and washed with water and brine. The organic layers were dried with anhydrous magnesium sulfate, filtered, and solvent was evaporated. The crude product was chromatographed on 20 x 120 mm silica gel eluting sequentially with 1:9, 1:8, 1:7, 1:5, 1:3, and 1:1 ethyl acetate-hexane to afford 0.473 g (66%) of ketone **22** as a mixture of *syn* and *anti* diastereomers in a 1:1 ratio. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 5.08 (bd s, 1H, NH amide), 4.58-4.54 (m,

1H, CH cyclopropyl NO_2), 4.48-4.39 (m, 1H, CH amide), 3.14 (m, 1H, CH cyclopropyl-ketone), 2.08-2.07 and 1.71-1.69 (m, 2H, CH_2 cyclopropyl), 1.50-1.31 (m, 12H, CH_3's). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 204.3 (C=O cyclopropyl), 155.3 (C=O Boc), 80.1 (4° C Boc), 60.7 (CH cyclopropyl NO_2), 56.0 (CH-NH), 28.3 (CH_3's Boc), 27.8 (CH cyclopropyl-ketone), 18.6 (CH_2 cyclopropyl), 16.6 (CH_3-CH). Mass spectrum (ESI-MS) *m*/*z* ($C_{11}H_{18}N_2O_5$) calculated for (M+1) 259.1216, found 259.1288.

***tert*-Butyl (2R)-1-hydroxy-1-(2-nitrocyclopropyl)propan-2-ylcarbamate 23.** To a solution of Boc-ala-nitro ketone **22** (0.463 g, 1.79 mmol) in 13 mL of methanol was added sodium borohydride (0.341 g, 8.79 mmol). The reaction was stirred at room temperature for one hour. The solution was then diluted with ethyl acetate and washed three times with deionized water. The organic layer was dried with anhydrous magnesium sulfate, filtered and the solvent was evaporated. The crude product was chromatographed on 20 x 120 mm silica gel eluting sequentially with 1:6, 1:5, 1:4, 1:2, and 1:1 ethyl acetate-hexane to afford of Boc-nitro alcohol **23** as a mixture of isomers. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 4.87-4.82 (bs, 1H, NH amide), 4.39-4.30 (m, 1H, CH propyl-NO_2), 4.26-4.24 (m, 1H, CH-NH), 3.82-3.75 (m, 1H, CH-OH), 3.66 (m, 1H, CH propyl-CH-OH), 2.02-2.01 and 1.78-1.76 (m, 2H, CH_2 propyl), 1.42-1.40 (m, 9H, CH_3's Boc), 1.20-1.16 (m, 3H, CH_3 CH-NH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157.1 (C=O Boc), 80.6 (4° C Boc), 71.9 (CH-OH), 60.7 (CH propyl-NO_2), 51.5 (CH-NH), 28.4 (CH_3 Boc), 26.7 (CH propyl-CH-OH), 16.0 (CH_3-CH-NH), 14.1 (CH_2 propyl). Mass spectrum (ESI-MS) *m*/*z* ($C_{11}H_{20}N_2O_5$) calculated for (M+1) 261.1372, found 261.1445.

***tert*-Butyl (2R)-1-(2-aminocyclopropyl)-1-hydroxypropan-2-ylcarbamate 24**. To a solution of Boc-nitro-alcohol (0.0544 g, 0.209 mmol) in 6 mL of methanol was

added ammonium formate (0.421 g, 6.69 mmol) followed by 50.0 mg of 10% palladium on carbon catalyst and the mixture was stirred for 20 minutes at room temperature. The reaction mixture was filtered, washing with methanol and the solvent was evaporated to give 340 mg of solid. The solid was then dissolved in 20 mL of deionized water and 1 M hydrochloric acid was added to acidify the solution to a pH of 1. The solution was then extracted with ethyl acetate and the aqueous layer was lyophilized to give 120 mg of crude amine **24**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.78-3.64 (m, 1H, C<u>H</u>-NH), 3.46-3.08 (m, 1H, C<u>H</u>-OH), 2.80-2.76 (m, 1H, C<u>H</u> cyclopropyl NH_2), 1.47 (s, 9H, C<u>H$_3$</u>'s Boc), 1.40-1.41 (m, 1H, C<u>H</u> propyl-CH-OH), 1.22-1.20 (m, 3H, C<u>H$_3$</u>-CH-NH), 1.18-1.01 (m, 2H, C<u>H$_2$</u> propyl). ^{13}C-NMR (125 MHz, $CDCl_3$) δ 157 (C=O Boc), 81.3 (4°<u>C</u> Boc), 71.8-71.6 (<u>C</u>H-OH), 51.5 (<u>C</u>H propyl-NH_2), 50.7 (<u>C</u>H-NH), 28.3 (<u>C</u>H$_3$'s Boc), 19.3-15.3 (<u>C</u>H propyl-CH-OH), 12.5 (<u>C</u>H$_3$-CH-NH), 9.1-8.3 (<u>C</u>H$_2$ propyl). Mass spectrum (ESI-MS) *m/z* ($C_{11}H_{22}N_2O_3$) calculated for (M+1) 231.1630, found 231.1703.

Synthetic method for coupling.

(R)-N-benzyl-4-oxooxetane-2-carboxamide 25. To a solution of β-lactone (**12**) (0.058 g, 0.50 mmol) in 1.5 mL methylene chloride was added EDCI (0.96 g, 0.50 mmol) and reaction was stirred for 25 minutes at room temperature. After 25 minutes benzyl amine (35.7 µL, 0.33 mmol) in 0.5 mL of methylene chloride was added followed by triethyl amine (0.46 mL, 3.3 mmol) and reaction was allowed to stir for 24 hours at room temperature. The reaction mixture was then poured into 2 mL 1M HCl and extracted two times with ethyl acetate. The organic layers were combined and washed with deionized water, dried with anhydrous magnesium sulfate, and solvent was evaporated. The crude

product was purified on 20 x 120 mm silica gel eluting sequentially 1:2, 1:1, 2:1, 4:1, and 5:1 ethyl acetate-hexane, followed by 100% ethyl acetate and 100% methanol washes to give 25.0 mg of a coupled product. It is not certain that the product isolated was **25**.

Synthetic methods for Cbz-Alanine analog.

(R)-benzyl 1-(methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate 27. To a solution of N-[(Benzyloxy)carbonyl]-D-alanine (1.00 g, 4.48 mmol) in 17 mL of methylene chloride at -10°C, was added N, O-dimethyl hydroxyl amine hydrochloride (0.44 g, 4.48 mmol), N-methyl morpholine (0.50 mL, 4.48 mmol), followed by five portions of EDCI (0.86 g, 4.48 mmol) over thirty minutes. The solution was allowed to stir overnight at room temperature. The solution was poured into 5 mL of ice cold 1 M hydrochloric acid and the aqueous layer was washed two times with methylene chloride. The organic layers were combined, washed with saturated sodium bicarbonate, and dried with anhydrous magnesium sulfate. The solution was filtered and solvent was evaporated to give 1.13 g of Weinreb amide **27** (95%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35-7.25 (m, 5H, phenyl H's Cbz), 5.57 (bs, 1H, NH), 5.12-5.08 (m, 2H, CH_2 Cbz), 4.74 (m, 1H, CH-NH), 3.77 (s, 3H, CH_3-O), 3.20 (s, CH_3-N), 1.33 (CH_3-CH-NH).

(R)-benzyl 3-oxopent-4-en-2-ylcarbamate 28. To a solution of Weinreb amide (**26**) (0.558 g, 2.10 mmol) in 7.2 mL of tetrahydrofuran (THF) under argon and on ice was added vinyl magnesium bromide (7.2 mL, 6.29 mmol). After 10 minutes, the ice bath was removed and the solution was allowed to stir for two hours at room temperature. The solution was diluted with ethyl acetate and the organic layer was washed with 1M HCl and brine. The combined organic layers were dried with anhydrous magnesium

sulfate, filtered and the solvent was evaporated. Purification on silica gel with the CombiFlash system afforded 0.1579 g (32%) of pure enone **28**. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.35-7.32 (m, 5H, phenyl H's Cbz), 6.44-6.40 (m, 1H, C<u>H</u>=CH_2), 5.90-5.78 (m, 2H, CH=C<u>H</u>$_2$), 5.12-5.07 (s, 2H, C<u>H</u>$_2$ Cbz), 4.68-4.65 (m, 1H, C<u>H</u>-NH), 1.36-1.26 (d, 3H, C<u>H</u>$_3$-CH-NH).

Benzyl (R)-1-(2-nitrocyclopropyl)-1-oxopropan-2-ylcarbamate 29. To a solution of enone (**28**) (0.1942 g, 0.833 mmol) in 4 mL methylene chloride on ice was added crushed potassium carbonate (0.1150 g, 0.833 mmol) and one equivalent of bromonitromethane (58.1 μL, 0.833 mmol). After 30 minutes the ice bath was removed and two more equivalents of bromonitromethane was added at two hour intervals. After a total of six hours, the reaction solution was diluted with ethyl acetate, and washed with water and brine. The organic layers were dried with anhydrous magnesium sulfate, filtered and solvent was evaporated. The crude product was purified by flash chromatography on the CombiFlash system to afford 170 mg (69%) of pure nitro cyclopropyl ketone **29** as a mixture of *syn* and *anti* diastereomers in a 1:1 ratio. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.36-7.34 (m, 5H, phenyl H's Cbz), 5.45-5.44 (m, 1H, N<u>H</u>), 5.10-5.07 (s, 2H, C<u>H</u>$_2$ Cbz), 4.67-4.53 (m, 2H, C<u>H</u>-NH and C<u>H</u> propyl-NO_2), 3.10-3.08 (m, 1H, C<u>H</u> propyl-C=O), 2.06-2.03 and 1.70-1.68 (m, 2H, C<u>H</u>$_2$ propyl), 1.44-1.41 (m, 1H, C<u>H</u>$_3$-CH-NH).

CHAPTER THREE: RESULTS, DISCUSSION, AND CONCLUSION

This project was an attempt to make analogs of the natural product belactosin A, which includes L-alanine coupled to a *trans*-cyclopropyl amine that forms an amide bond to a β-lactone. The goals of this synthesis included the synthesis of a simple β-lactone starting from L-malic acid as well as the synthesis of two alanine analogs of belactosin, one of which included a hydroxyl component and one without. There were several issues in the scope of this project including the stability of the β-lactone, the coupling step, the synthesis of the thioacetal, as well as the protecting group on the "left side of the molecule.

β-Lactone

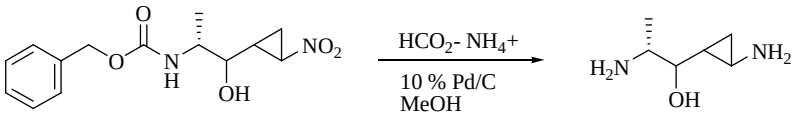
The β-lactone stability was a concern from the beginning of the synthesis, since these compounds were known to polymerize.[13, 14, 16] During the synthesis of compound **11**, it was observed that if the color of the mixture changes to dark red the compound polymerized during that step, but if the mixture stays pale yellow then polymerization did not occur. Many of the compounds in this series have the ability to polymerize. For example, degradation occurred once during formation of compound **10**, shown in Figure 16.

The second observation about the β-lactone is inconsistency in amine coupling. While a product was isolated in the coupling of benzyl amine to β-lactone **12**, no product was isolated in the coupling of cyclopropyl amine **24** to **12**. In the coupling of **12** to

benzyl amine the product that was isolated showed by NMR data that the amine did couple to a compound, however the peaks that should have been seen for the β-lactone were absent in the spectra. The benzyl amine could have coupled during the coupling reaction to several things. It is possible that the β-lactone opened and started polymerizing in the beginning of the reaction causing the benzyl amine to bid to a dimer or trimer of the polymer product instead of the β-lactone **12.** In the case of the coupling of the amine **24**, the products that were isolated each showed polymerization as well as possible decomposition within the ^{1}HNMR spectrum. With the amine **24** coupling, a possible problem was the solubility of the amine, since it did not seem to dissolve during the 17-hour reaction. However, it is also possible that the β-lactone polymerized under the reaction conditions. It was observed that over a period of about 6 months the benzyl protected β-lactone decomposed over time. It is possible that this β-lactone **12** will need to be synthesized and then used immediately after synthesis to prevent the compound from decomposing.

Analogs

Several challenges were encountered in the synthesis of belatosin A analogs. The main change in strategy was the protecting group that was used on the "left side" of the molecule. The initial protecting group was Cbz. However the reduction step removed the Cbz protecting group and reduced the nitro group to give compound **32** as shown in Figure 21. Since the product was a diamine, there was no longer control of the selectivity in the coupling step.

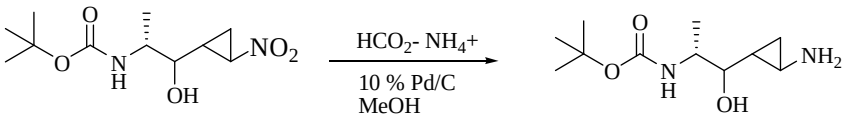


31 **32**

Figure 21: Reduction step of Cbz analog.

Since the Cbz group was also reduced during the reduction step, it was realized that the protecting group needed to be changed. The protecting group was changed to Boc protecting group since some of the Boc-D-ala enone (**21**) was already synthesized in the lab.

The Boc protecting group in **24** allowed for reduction of the nitro to afford a cyclopropyl amine **25** for coupling to the "right side" of the molecule, as shown below in Figure 22.



24 **25**

Figure 22: Reduction step of Boc analog.

From previous results in the Dunlap lab, it has been shown that the cyclopropyl amine will couple to form several amides, as shown in Figure 23. Both mono-amides as well as a diamide are possible in the coupling step as shown in Figure 23. The results are inconclusive as to which mono amide was isolated in the lab, but it is known from mass spectroscopy data that the diamide is formed in the coupling step.

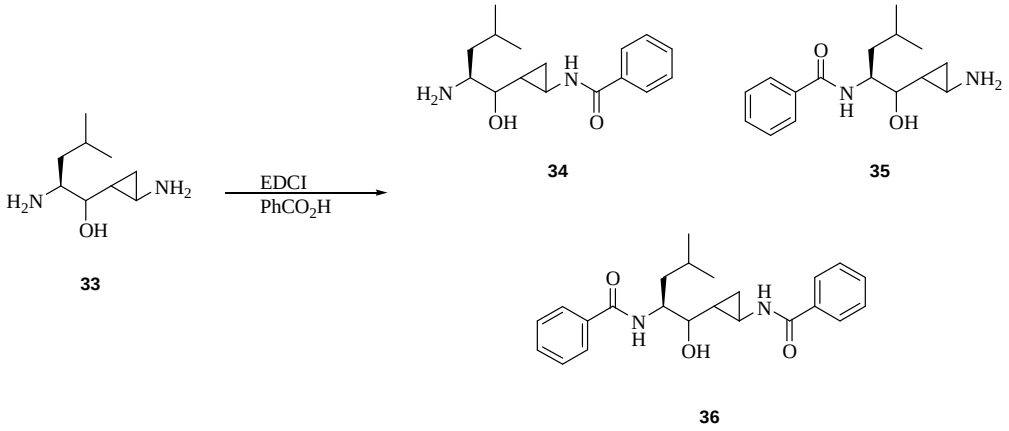


Figure 23: Cyclopropyl amine coupling.

Although the Boc protecting group solved the problem of double reduction, removal of the Boc group involves acidic conditions, which can be incompatible with the cyclopropyl group. In the presence of acid, the hydroxyl group to the right of the cyclopropyl could protonated, which would cause the alcohol to become a good leaving group in the form of water creating a carbocation next to the cyclopropyl ring. The molecule could then rearrange to open the cyclopropyl or rearrange into a four membered ring shown below in Figure 24.[17] The name of the mechanism of this rearrangement is known as a cyclopropylcarbinyl rearrangement. The way this mechanism works is a carbocation is formed next to the cyclopropyl ring, which is already strained, so in order to relieve the strain in the cyclopropyl ring the system will undergo a rearrangement.[17]

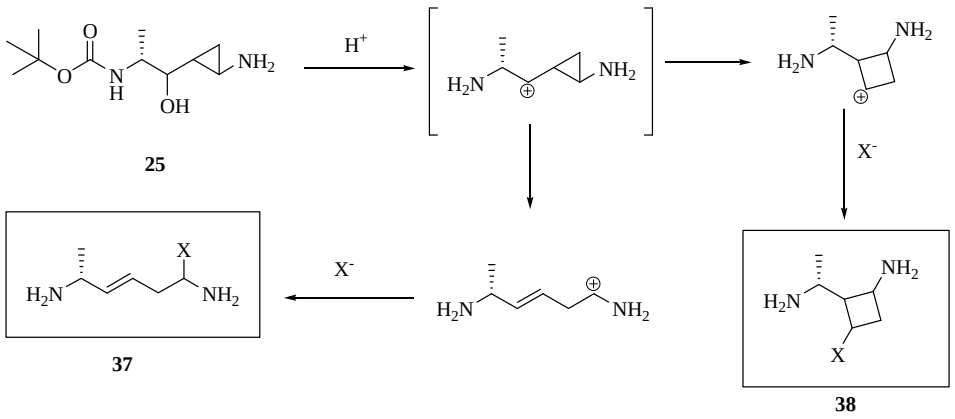


Figure 24: Removal of Boc protecting group and opening cyclopropane

It is possible that this problem could be eliminated by the removal of the hydroxyl component. While there are methods for removing an alcohol group, most involve radical reactions that can cause the cyclopropyl ring to open. A solution is to deal with the ketone of the alcohol instead of the alcohol itself. There are a couple of methods of reducing a ketone to a methylene group. The first is to convert the ketone into a thioacetal and then reduce the thioacetal with Raney nickel. A second method is the Wolff Kishner reduction, which involves condensation of hydrazine with a ketone to form a hydrazone group which leaves as nitrogen gas under basic conditions.

Thioacetal Synthesis

Several attempts and methods were made to form a thioacetal with the Cbz-ketone (**29**). The first attempt to making the thioacetal, compound **14** was reacted with 1, 2 ethanedithiol with a catalytic amount of p-toluenesulfonic acid as shown in Figure 25.[18] This reaction was set up in toluene and was refluxed at 110°C, which resulted in the

decomposition of the ketone **14**. Once it was discovered that the materials were breaking down at the higher temperatures the method was changed to one that employed milder conditions.

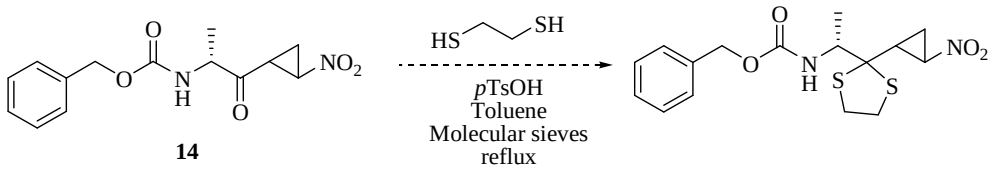


Figure 25: First attempt to synthesize thioacetal.

The second method that was tried was Kamal's method, which employed N-bromosuccimide (NBS) as a catalyst at room temperature.[15] In this reaction, as shown in Figure 26, compound **14** was reacted with 1,2-ethanedithiol and a 15 mol % of NBS in dichloromethane was allowed to stir overnight at room temperature.

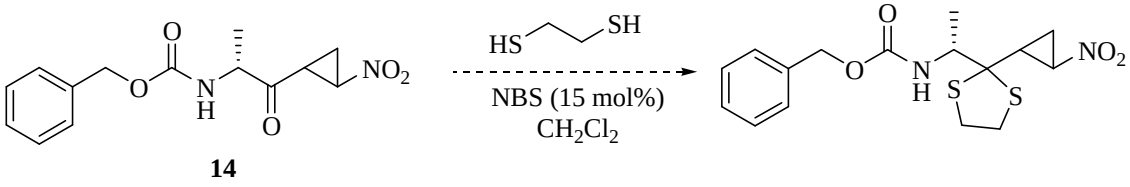


Figure 26: Second attempt to synthesize thioacetal.

The result of this reaction was that the unreacted starting material was all that was isolated. The possible problems with this reaction may be that more catalyst needed to be used or the fact that the reaction needed to go for a longer time. This method is specifically for aldehydes, which are more reactive than ketones, which could have been

the problem. It was decided then to change the solvent to chloroform and reflux at a low temperature.

The third attempt to make the thioacetal included changing the solvent from dichloromethane to chloroform so that the reaction could reflux at 61°C as well as changing the amount of catalyst used from 15 mol% to 45 mol%. The results were inconclusive. There were NMR signals in the region of the spectrum where methylene protons adjacent to the sulfur would be expected (δ 3.1-3.3 ppm), but the integration as of the peaks were not what would be expected.[19] A possible solution is to use a different catalyst, namely 1,3-dibromo-5,5-dimethylhydantoin (DBH) which is known to convert carbonyls to thioacetals under mild conditions.[20] It is also possible that the ketone is too hindered to be converted into the thioacetal and, in that case, it may be better to attempt a Wolff-Kishner reduction.

Conclusions

Belactosin A is a natural product that has inhibitory effects on the 20S core particle of the 26S proteasome. Since belactosin A is too toxic for use in humans, analogs are needed to help with the efficacy of the drug. This research focused on trying to synthesize analogs of belactosin A. The goals of the project were to synthesize a simple β-lactone as well as synthesize two analogs of belactosin A, one with a hydroxyl component and one without. The research faced many challenges in the synthesis from the protecting group of the amino acid to the coupling of the β-lactone to the amine.

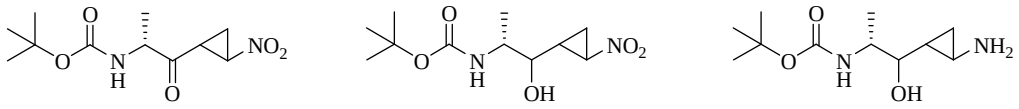
The synthesis of the β-lactone was completed in four steps that resulted in the formation of the simple β-lactone **12**. The β-lactone proved problematic in the attempts to couple. Since the results were inconclusive in the β-lactone's ability to couple to both benzyl amine and the Boc-amine **25**, more research needs to is required in order to see if the β-lactone will work to synthesize analogs of belactosin A. It is possible that freshly synthesized β-lactone needs to be used in the coupling step, and this is one of the research goals that will need to be accomplished in the future.

Another issue that was addressed in this research was the protecting group on the amino acid. At the start of this project, the protecting group that was used was Cbz group. This group was reduced off during the transfer hydrogenation step of the nitro group in the synthesis. During the hydrogenation step, this group produced a diamine, which lead to problems arising in the coupling step of the synthesis. In previous research on the amino acid leucine showed that the coupling step of a diamine produced not only the diamide **36** but also produced two different monoamides **34** and **35**. In short, this protecting group gave no selectivity in which side of the molecule would couple to the β-lactone.

Since it was figured out early that Cbz protected amino acids were going to be a problem the focus shifted on these compounds to see if we could make a thioacetal in order to ultimately remove the hydroxyl group. Several attempts were made to try to synthesize the thioacetal, but all were either inconclusive or failed. Future research is needed in this area to see if it is possible to remove the hydroxyl group on the analog. Many avenues can be utilized to try to remove the ketone or the alcohol group that have

yet to be explored including more attempts to make the thioacetal as well as a Wolff-Kishner reduction.

Since the protecting group Cbz was too reactive under hydrogenation condition, and that Boc-protected amino acid was already available, the latter group was used in the synthesis. This particular series produced three new compounds (**22-24**) that were previously uncharacterized as shown below in Figure 27. The Boc protecting group gives selectivity for coupling to the amine whereas there was no selectivity possible when Cbz was reduced off.



22 **23** **24**

Figure 27: Newly synthesized compounds.

Even though the Boc-protected amine would allow for selectivity in the coupling of the β-lactone to the "right side" of the molecule, coupling to the "left side" of the molecule would prove to be difficult, since the removal of Boc requires acidic conditions. Acidic conditions can possibly cause the opening or rearrangement of the cyclopropyl group, which is why the focus of the Cbz series switched to the removal of the hydroxyl group. The removal of the hydroxyl group would help to keep cyclopropyl group from opening in acidic conditions, since the alcohol would not be there to protonate and leave a carbocation.

There are many areas of this research that can be expanded upon. Some future work includes finding the best protecting group for use in the preparation of analogs, more attempts at removal of the ketone, as well as more research on the coupling step using the simple β-lactone that was synthesized. Once these areas are explored in more depth, more analogs of belactosin A can be synthesized. Once analogs are synthesized, a full range of biological testing can then be done to see if they have biological activity.

WORKS CITED

1. Sherr, C. J. Cancer Cell Cycles. *Science.* **1996**, *274*, 1672-1677.

2. Kamb, A.; Wee, S.; Lengauer, C. Why is cancer drug discovery so difficult? *Nat. Rev. Drug Discov.* **2006**, *6*, 115-120.

3. Weathington, N. M.; Mallampalli, R. K. Emerging therapies targeting the ubiquitin proteasome system in cancer. *J. Clin. Invest*. **2014**, *124* 6-12.

4. Dick, L. R.; Fleming, P. E. Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy. *Drug Discov. Today.* **2010**, *15*, 243-249.

5. Steele, J. M. Carfilzomib: A new proteasome inhibitor for relapsed or refractory multiple myeloma." *J. Oncol. Pharm. Pract.* **2013**, *19*, 348-354.

6. da Fonseca, P. C. A.; He, J.; Morris, E. P. 'Molecular Model of the Human 26S Proteasome." *Mol. Cell.* **2012**, *46*, 54-66 and references therein.

7. Asai, A.; Hasegawa, A.; Ochiai, K.; Yamashita, Y.; Mizukami, T. Belactosin A, a Novel Antitumor Antibiotic Acting on Cyclin/CDK Mediated Cell Cycle Regulation, Produced by *Streptomyces* sp. *J. Antibiot.* **2000**, *53*, 81-83.

8. Armstrong, A. and Scutt, J. N. Total synthesis of (+)-belactosin A. *Chem. Commun.* **2004**, *5*, 510-511.

9. Kawamura, S.; Unno, Y.; List, A.; Mizuno, A.; Tanaka, M.; Sasaki, T.; Arisawa, M.; Asai, A.; Groll, M.; Shuto, S. Potent Proteasome Inhibitors Derived from the Unnatural cis-Cyclopropane Isomer of Belactosin A: Synthesis, Biological Activity, and Mode of Action. *J. Med. Chem.* **2013**, *56*, 3689-3700.

10. Vagner, J.; Qu, H; Hruby, V. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292-296.

11. Dunlap, N. K.; Basham, J.; Wright, M.; Smith, K.; Chapa, O.; Huang, J.; Shelton, W.; Yatsky, Y. Synthesis of nitrocyclopropyl peptidomimetics. *Tetrahedron Lett.* **2013,** *54*, 6596-6598 and references therein.

12. Dunlap, N.; Lankford, K. R.; Pathiranage, A. L.; Taylor, J.; Reddy, N.; Gouger, D.; Singer, P.; Griffin, K.; Reibenspies J. Three step synthesis of cyclopropyl peptidomimetics. *Org. Lett.* **2011**, *13*, 4879-4881.

13. Cammas, S.; Renard, I.; Boutault, K.; Guérin, P. A Novel Synthesis of Optically Active 4-Benzyloxy- and 4-Alkyloxycarbonyl-2-Oxentanones. *Tetrahedron: Asymmetry*. **1993**, *4*, 1925-1930.

14. Leboucher-Durand, M.; Langlois, V.; Guérin, P. 4-Carboxy-2-oxetanone as a new chiral precursor in the preparation of functionalized racemic or optically active poly(malic acid) derivatives. *Polym. Bull.* **1996**, *36*, 35-41.

15. Kamal, A. Mild and Efficient Chemoselective Protection of Aldehydes as Dithioacetals Employing N-Bromosuccinimide. *Synlett*. **2002**, *3*, 474-476.

16. Cammas, S.; Renard, I.; Langlois, V.; Guérin, P. Poly(β-malic acid): obtaining high molecular weights by improvement of the synthesis route. *Polymer*. **1996**, *37*, 4215-4220.

17. Wong, H. N. C.; Hon, M.; Tse C.; Yip, U.; Tanko, J.; Hudlicky, T. Use of Cyclopropanes and Their Derivatives in Organic Synthesis. *Chem. Rev.* **1989**, *89*, 165-198.

18. Fieser, L. F. Preparation of Ethylenethioketals. *J. Am. Chem. Soc.* **1954**, *76*, 1945-1947.

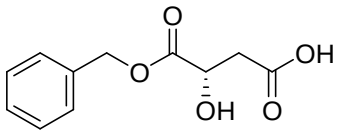
19. Sternson, L. A., Coviello, D. A., Egan, R. S. Nuclear Magnetic Resonance Study of the Conformation of 1,3-Dithiolanes. *J. Am. Chem. Soc.* **1971,** *93*, 6529-6532.

20. Veisi, H.; Amiri, M; Hamidian, A. H.; Malkootikhah, J.; Fatolahi, L.; Faraji, A.; Sedrpoushan, A.; Maleki, B.; Saremi, S. G.; Noroozi, M.; Bahadoori, F.; Veisi, S. 1,3-Dibromo-5,5-Dimethylhydantoin (DBH) as a mild and efficient catalyst for chemoselective thioacetalization of carbonyl compounds and dethioacetalization under mild conditions. *Phosphorus Sulfur Silicon Relat. Elem.* **2010**, *185*, 689-696.

APPENDICES

APPENDIX A: NMR DATA

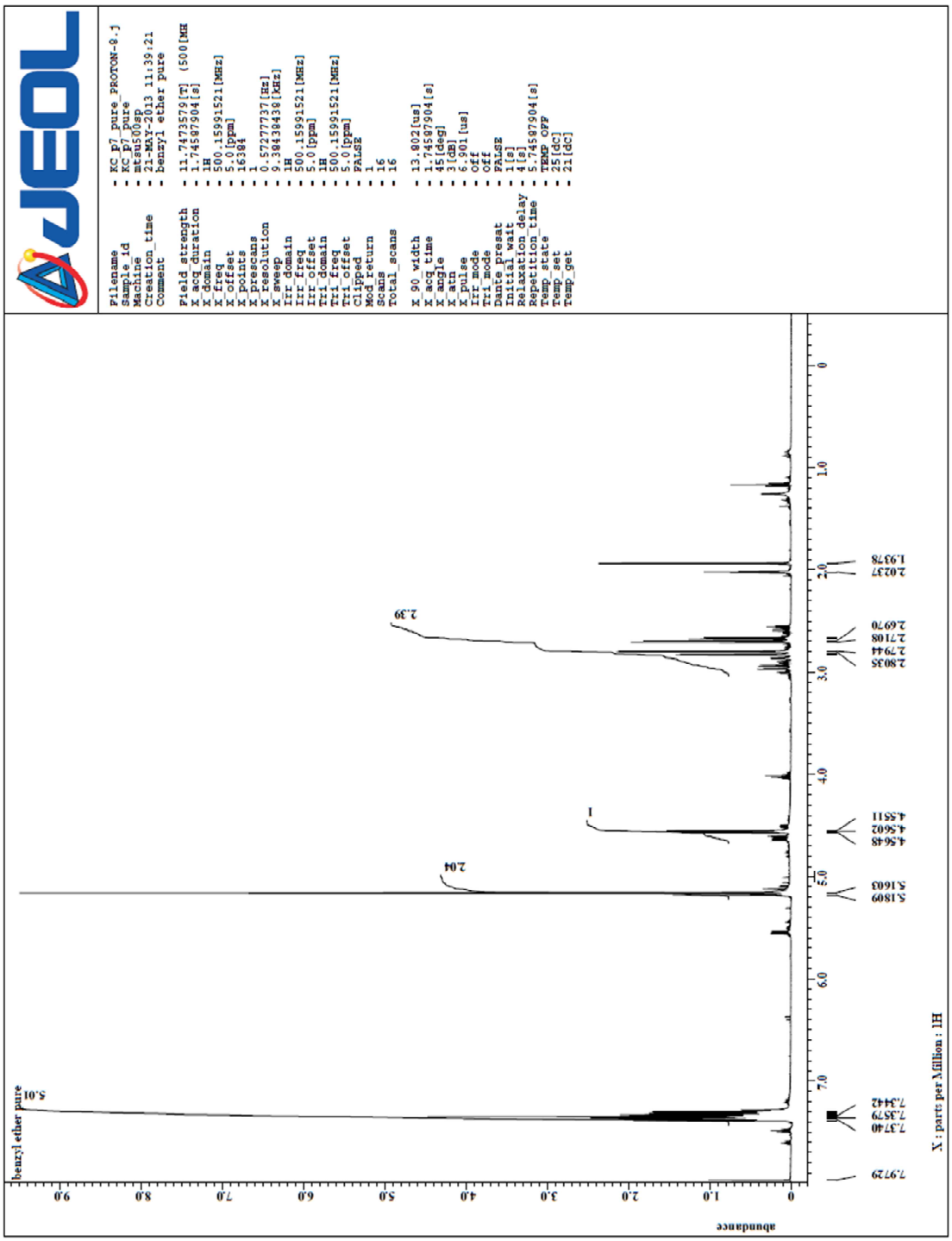
(3S)-4-benzyloxy-3-hydroxy-4-oxo-butanoic acid (10)

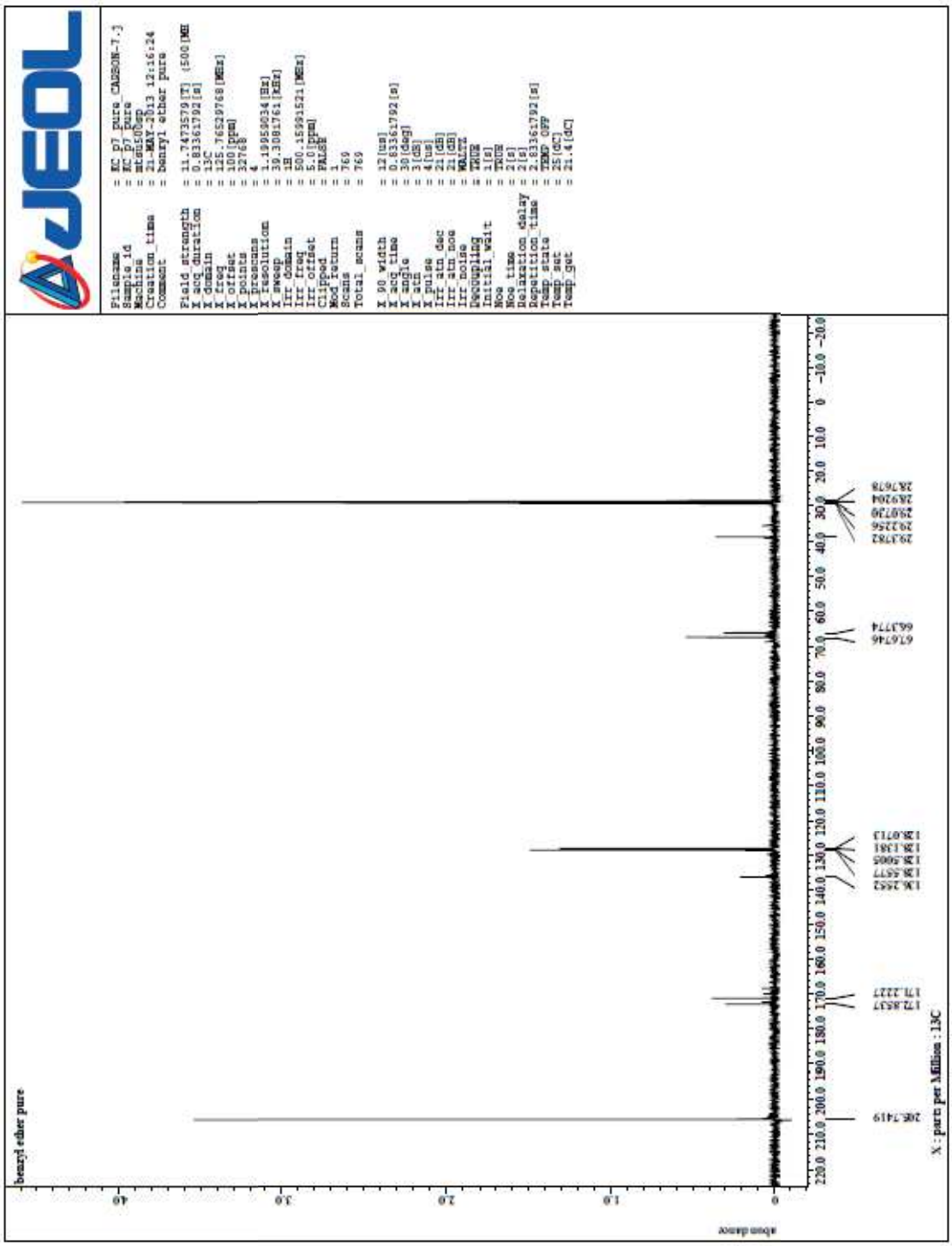


NMR

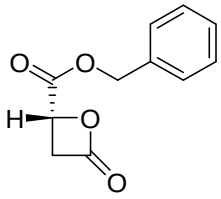
^{1}H

^{13}C


JEOL
Filename - KC_P7_pure_PROTON-0.j
Sample_id - KC_P7_pure
Machine - mtsu500sp
Creation_time - 21-MAY-2013 11:39:21
Comment - benzyl ether pure
Field_strength - 11.7473579[T] (500[MH
X_acq_duration - 1.74587904[s]
X_domain - 1H
X_freq - 500.15991521[MHz]
X_offset - 5.0[ppm]
X_points - 16384
X_prescans - 1
X_resolution - 0.57277737[Hz]
X_sweep - 9.38438438[KHz]
Irr_domain - 1H
Irr_freq - 500.15991521[MHz]
Irr_offset - 5.0[ppm]
Tri_domain - 1H
Tri_freq - 500.15991521[MHz]
Tri_offset - 5.0[ppm]
Clipped - FALSE
Mod_return - 1
Scans - 16
Total_scans - 16
X_90_width - 13.802[us]
X_acq_time - 1.74587904[s]
X_angle - 45[deg]
X_atn - 3[dB]
X_pulse - 6.901[us]
Irr_mode - Off
Tri_mode - Off
Dante_presat - FALSE
Initial wait - 1[s]
Relaxation delay - 4[s]
Repetition_time - 5.74587904[s]
Temp_state - TEMP OFF
Temp_set - 25[dc]
Temp_get - 21[dc]
benzyl ether pure
5.01
2.04
1
2.39
7.9729
7.3740
7.3579
7.3442
5.1809
5.1603
4.5648
4.5602
4.5511
2.8035
2.7944
2.7108
2.6970
2.0237
1.9378
abundance
X : parts per Million : 1H


JEOL
benzyl ether pure
X : parts per Million : 13C

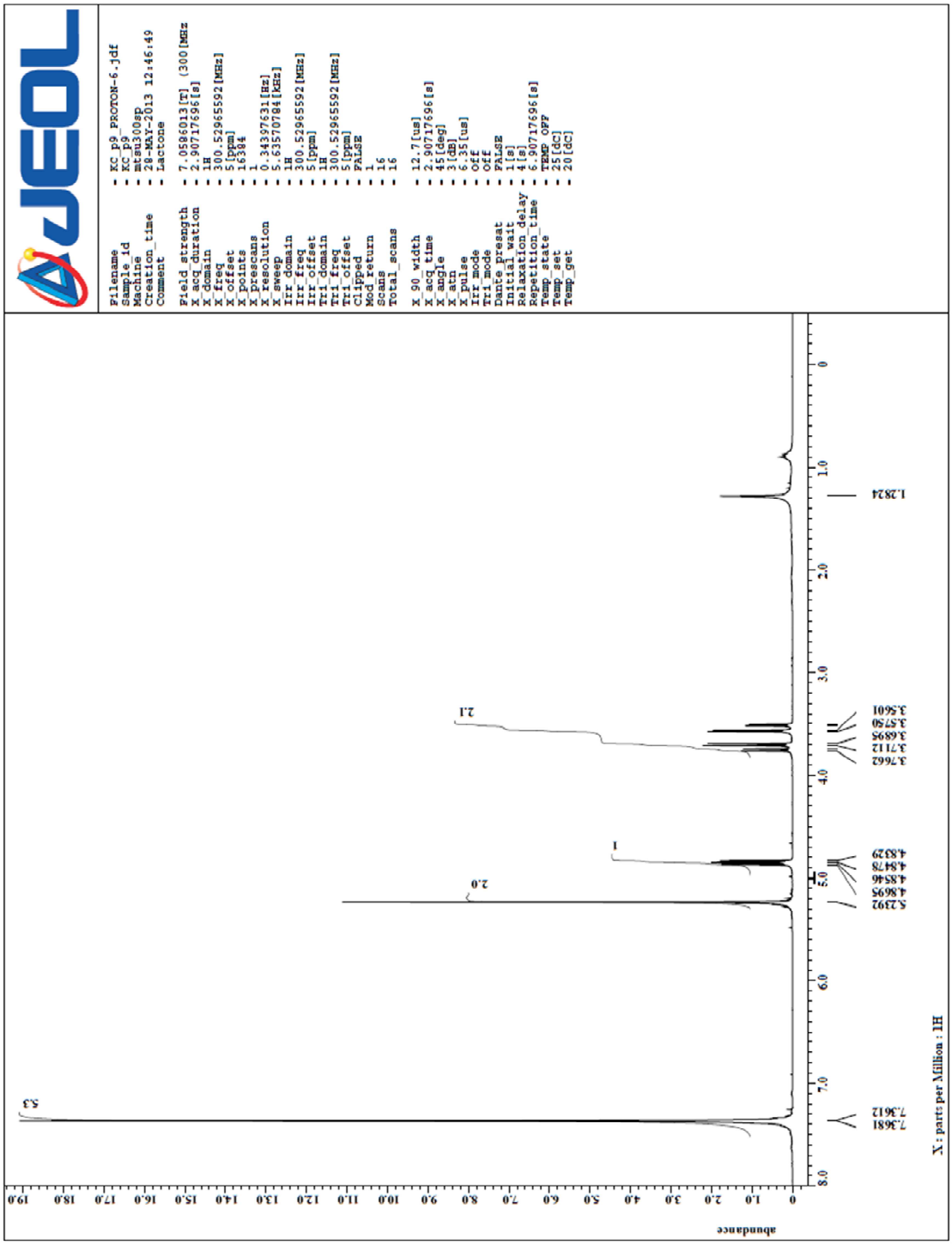
(R)-benzyl 4-oxooxetane-2-carboxylate (11)

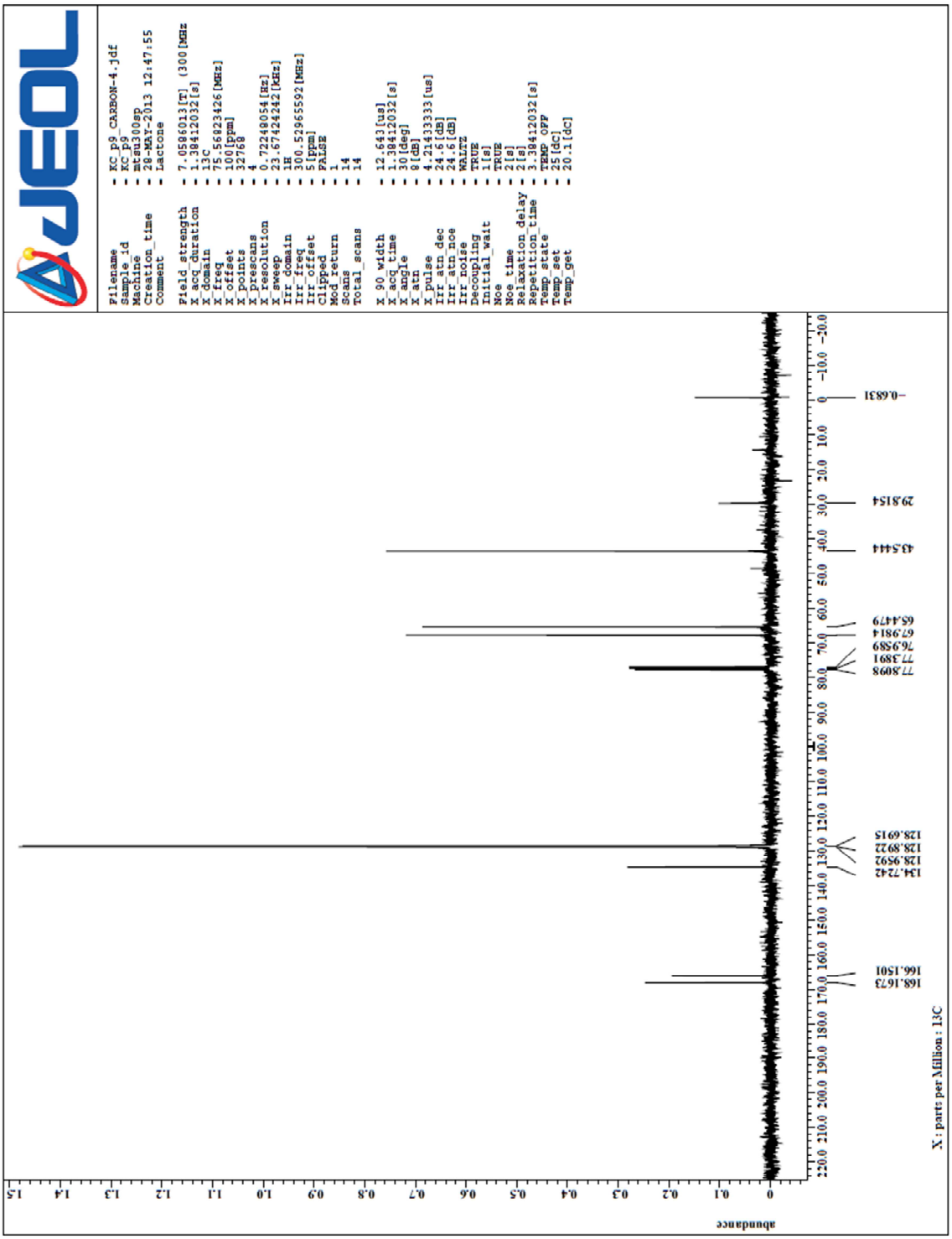


NMR

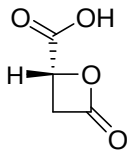
^{1}H

^{13}C


JEOL
Filename - KC_p9_PROTON-6.jdf
Sample_id - KC_p9
Machine - mtsu300sp
Creation_time - 28-MAY-2013 12:46:49
Comment - Lactone
Field_strength - 7.0586013[T] (300[MHz
X_acq_duration - 2.90717696[s]
X_domain - 1H
X_freq - 300.52965592[MHz]
X_offset - 5[ppm]
X_points - 16384
X_prescans - 1
X_resolution - 0.34397631[Hz]
X_sweep - 5.63570784[kHz]
Irr_domain - 1H
Irr_freq - 300.52965592[MHz]
Irr_offset - 5[ppm]
Tri_domain - 1H
Tri_freq - 300.52965592[MHz]
Tri_offset - 5[ppm]
Clipped - FALSE
Mod_return - 1
Scans - 16
Total_scans - 16
X_90_width - 12.7[us]
X_acq_time - 2.90717696[s]
X_angle - 45[deg]
X_atn - 3[dB]
X_pulse - 6.35[us]
Irr_mode - Off
Tri_mode - Off
Dante_presat - FALSE
Initial_wait - 1[s]
Relaxation_delay - 4[s]
Repetition_time - 6.90717696[s]
Temp_state - TEMP OFF
Temp_set - 25[dC]
Temp_get - 20[dC]
5.3
2.0
1
2.1
1.2824
3.7662
3.7112
3.6895
3.5750
3.5601
5.2392
4.8695
4.8546
4.8478
4.8329
7.3681
7.3612
abundance
X : parts per Million : 1H


JEOL
Filename - KC_p9_CARBON-4.jdf
Sample_id - KC_p9_
Machine - mtsn300sp
Creation_time - 28-MAY-2013 12:47:55
Comment - Lactone
Field_strength - 7.0586013[T] (300[MHz
X_acq_duration - 1.38412032[s]
X_domain - 13C
X_freq - 75.560223426[MHz]
X_offset - 100[ppm]
X_points - 32768
X_prescans - 4
X_resolution - 0.72248054[Hz]
X_sweep - 23.674242242[kHz]
Irr_domain - 1H
Irr_freq - 300.529655592[MHz]
Irr_offset - 5[ppm]
Clipped - FALSE
Mod_return - 1
Scans - 14
Total_scans - 14
X_90_width - 12.643[us]
X_acq_time - 1.38412032[s]
X_angle - 30[deg]
X_atn - 8[dB]
X_pulse - 4.21433333[us]
Irr_atn_dec - 24.6[dB]
Irr_atn_noe - 24.6[dB]
Irr_noise - WALTZ
Decoupling - TRUE
Initial_wait - 1[s]
Noe - TRUE
Noe_time - 2[s]
Relaxation_delay - 2[s]
Repetition_time - 3.38412032[s]
Temp_state - TEMP OFF
Temp_set - 25[dc]
Temp_get - 20.1[dc]
abundance
X : parts per Million : 13C
-0.6831
29.8154
43.5444
65.4479
67.9814
76.9589
77.3891
77.8098
128.6915
128.8922
128.9592
134.7242
166.1501
168.1673

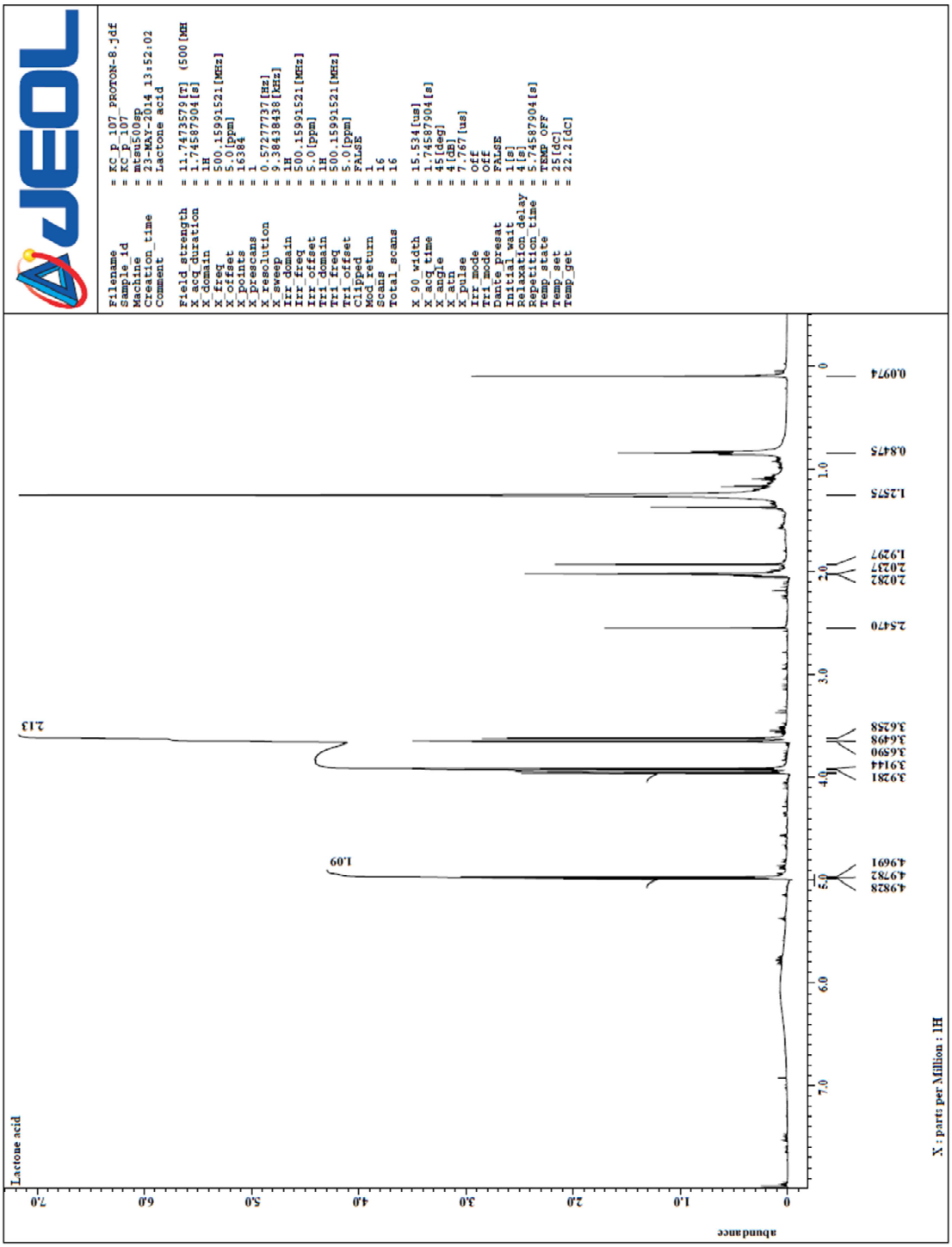
(2R)-4-oxooxetane-2-carboxylic acid (12)



NMR

^{1}H

^{13}C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Filename = KC_p_107_PROTON-8.jdf
Sample_id = KC_p_107_
Machine = mstu500gp
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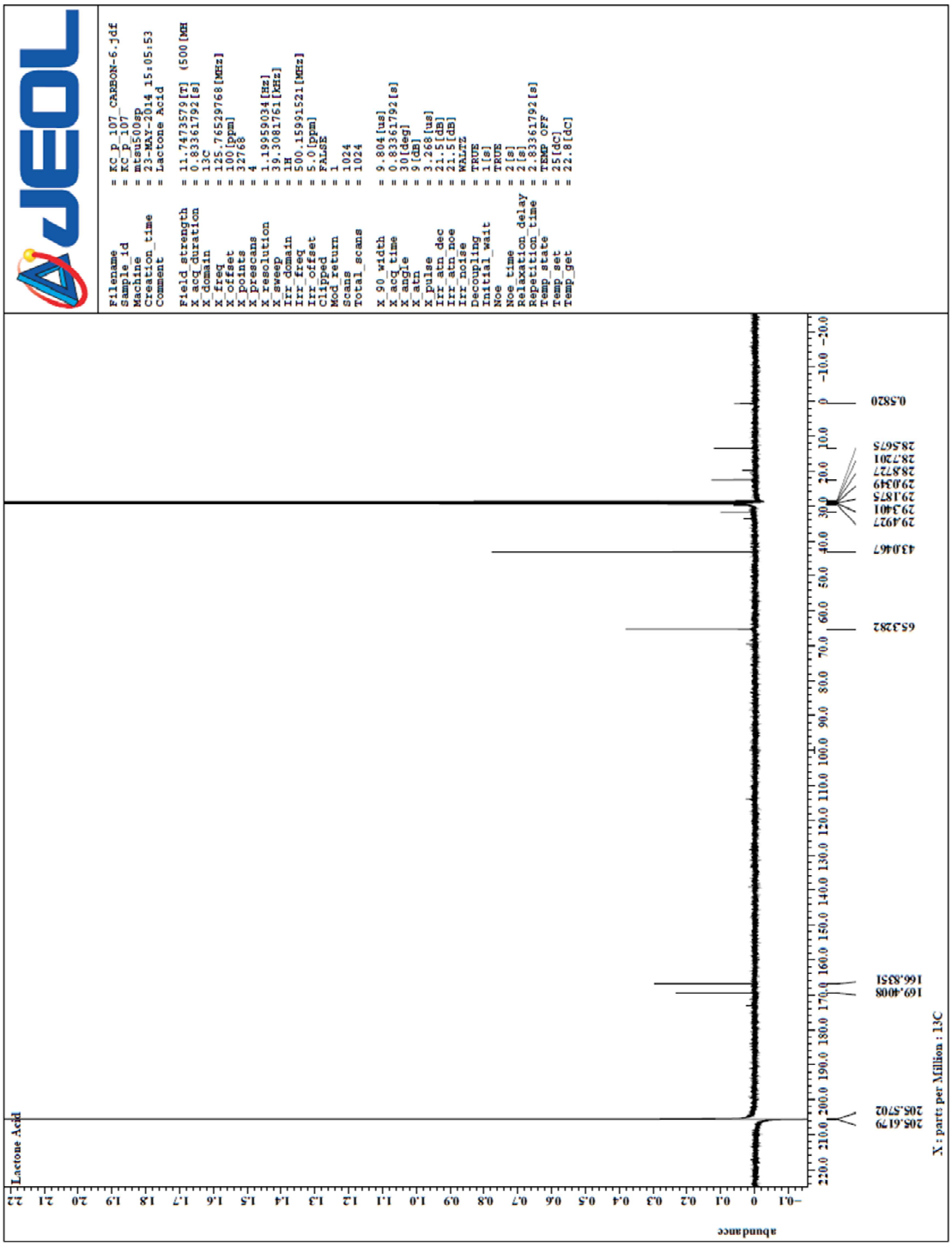
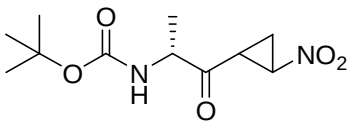
Comment = Lactone acid
Field strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[KHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_Return = 16
Scans = 16
Total_scans = 16
X_90_width = 15.534[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.767[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.2[dC]
Lactone acid
2.13
1.09
0.0974
0.8475
1.2575
1.9297
2.0237
2.0382
2.5470
3.6258
3.6498
3.6590
3.9144
3.9281
4.9691
4.9782
4.9828
abundance
X : parts per Million : 1H


JEOL
Filename = KC_p_107_CARBON-6.jdf
Sample_id = KC_p_107_
Machine = mtsu500sp
Creation_time = 23-MAY-2014 15:05:53
Comment = Lactone Acid
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.765297668[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.30817617[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 1024
Total_scans = 1024
X_90_width = 9.804[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.268[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dc]
Temp_get = 22.8[dc]
Lactone Acid
abundance
X : parts per Million : 13C
205.6179
205.5702
169.4008
166.8351
65.3282
43.0467
29.4927
29.3601
29.1875
29.0349
28.8727
28.7201
28.5675
0.5820

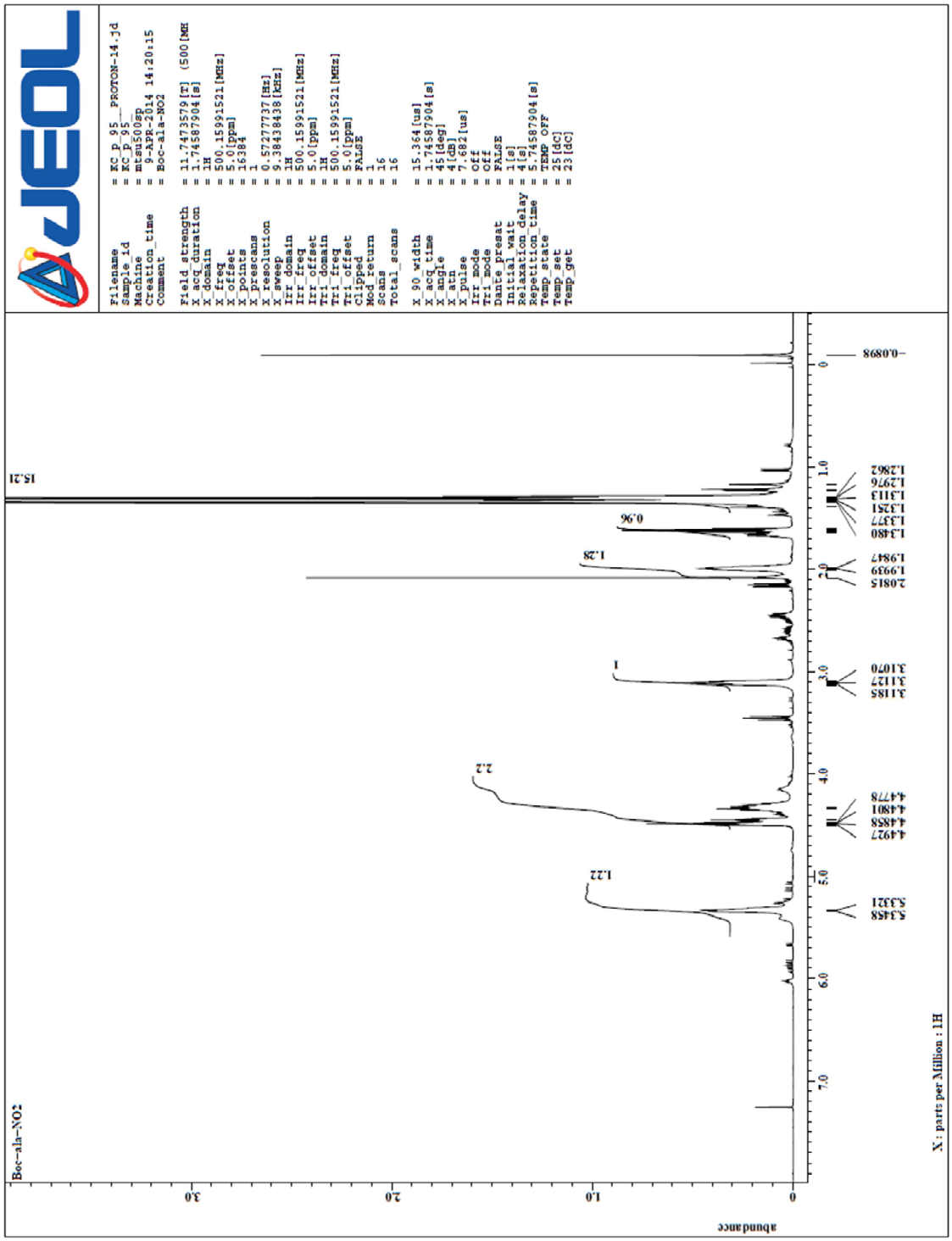
tert-Butyl-N-[(1R)-1-methyl-2-(2-nitrocyclopropyl)-2-oxo-ethyl]carbamate (22)

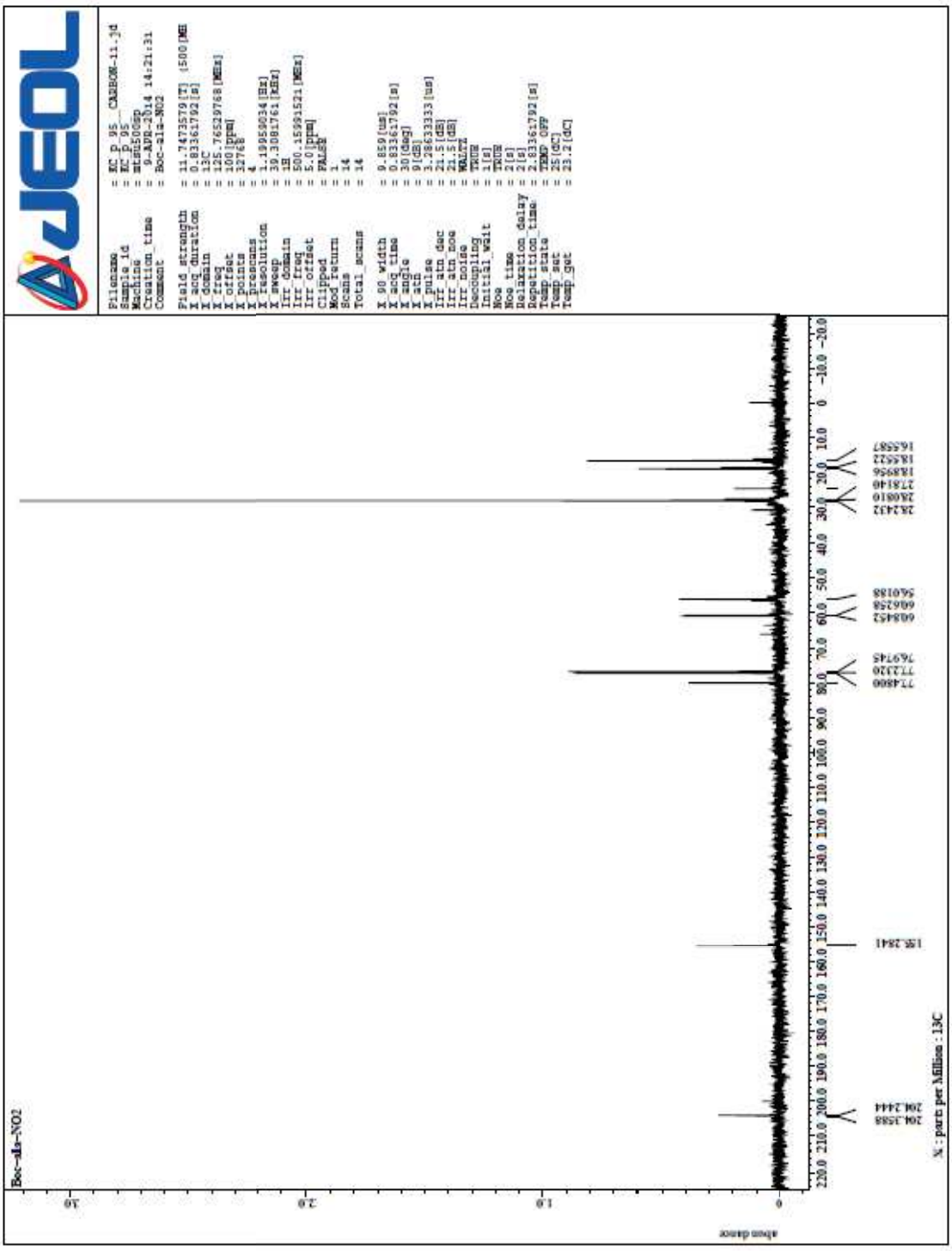


NMR:

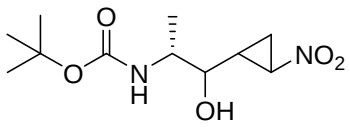
^{1}H-NMR

^{13}C-NMR


JEOL
Filename = RC_P_95__PROTON-14.jd
Sample_id = RC_P_95_
Machine = mteu500ap
Creation_time = 9-APR-2014 14:20:15
Comment = Boc-ala-NO2
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57227737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 16
Scans = 16
Total_scans = 16
X_90_width = 15.364[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.682[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 23[dC]
Boc-ala-NO2
15.21
1.28
0.96
1
2.2
1.22
-0.0898
5.3458
5.3321
4.4927
4.4858
4.4801
4.4778
3.1185
3.1127
3.1070
2.0815
1.9939
1.9847
1.3480
1.3377
1.3251
1.3113
1.2976
1.2862
abundance
X : parts per Million : 1H


JEOL
Boc-ala-NO2
arbitrary
% : parts per Million : 13C

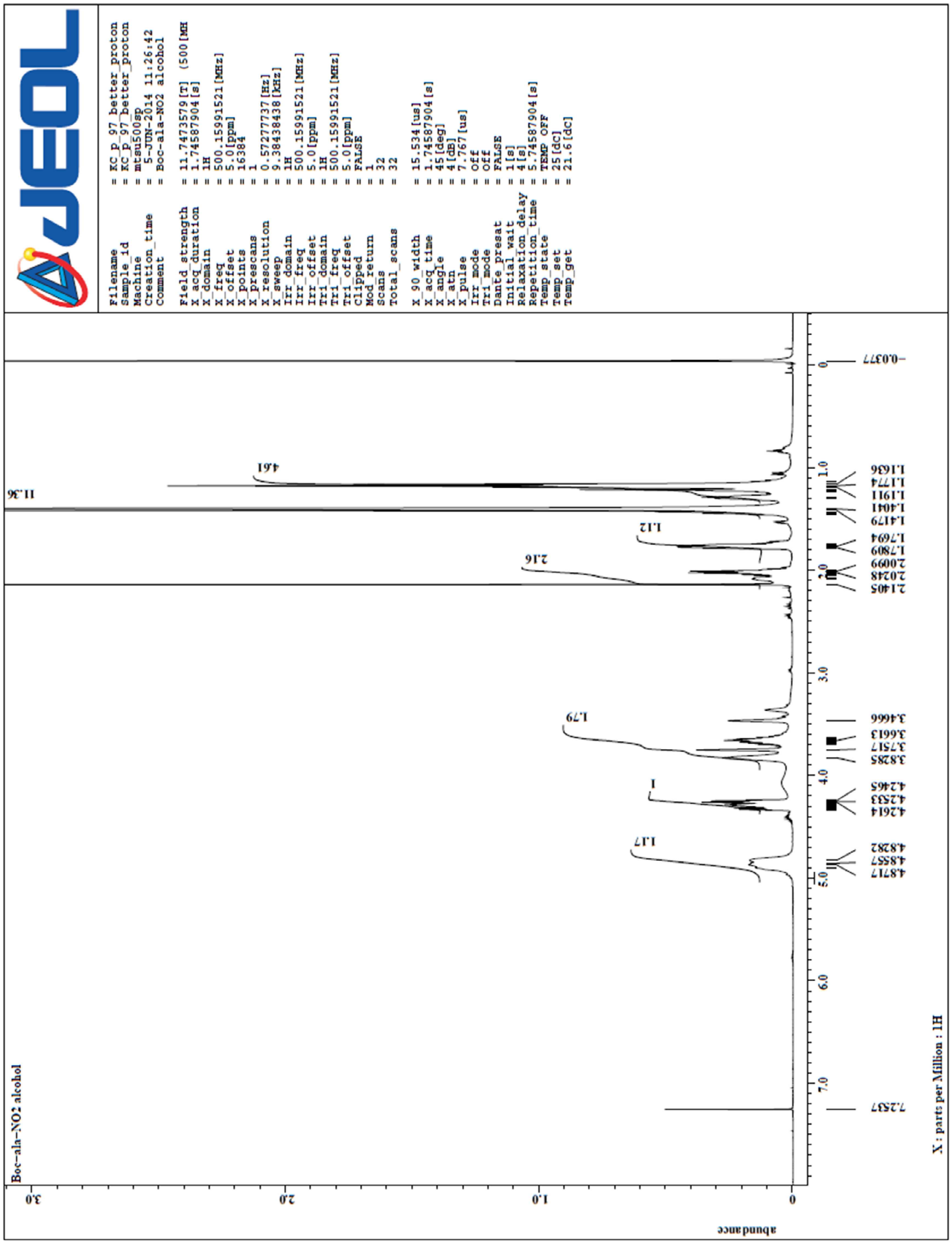
tert-Butyl-N-[(1R)-2-hydroxy-1-methyl-2-(2-nitrocyclopropyl)ethyl]-carbamate (23)

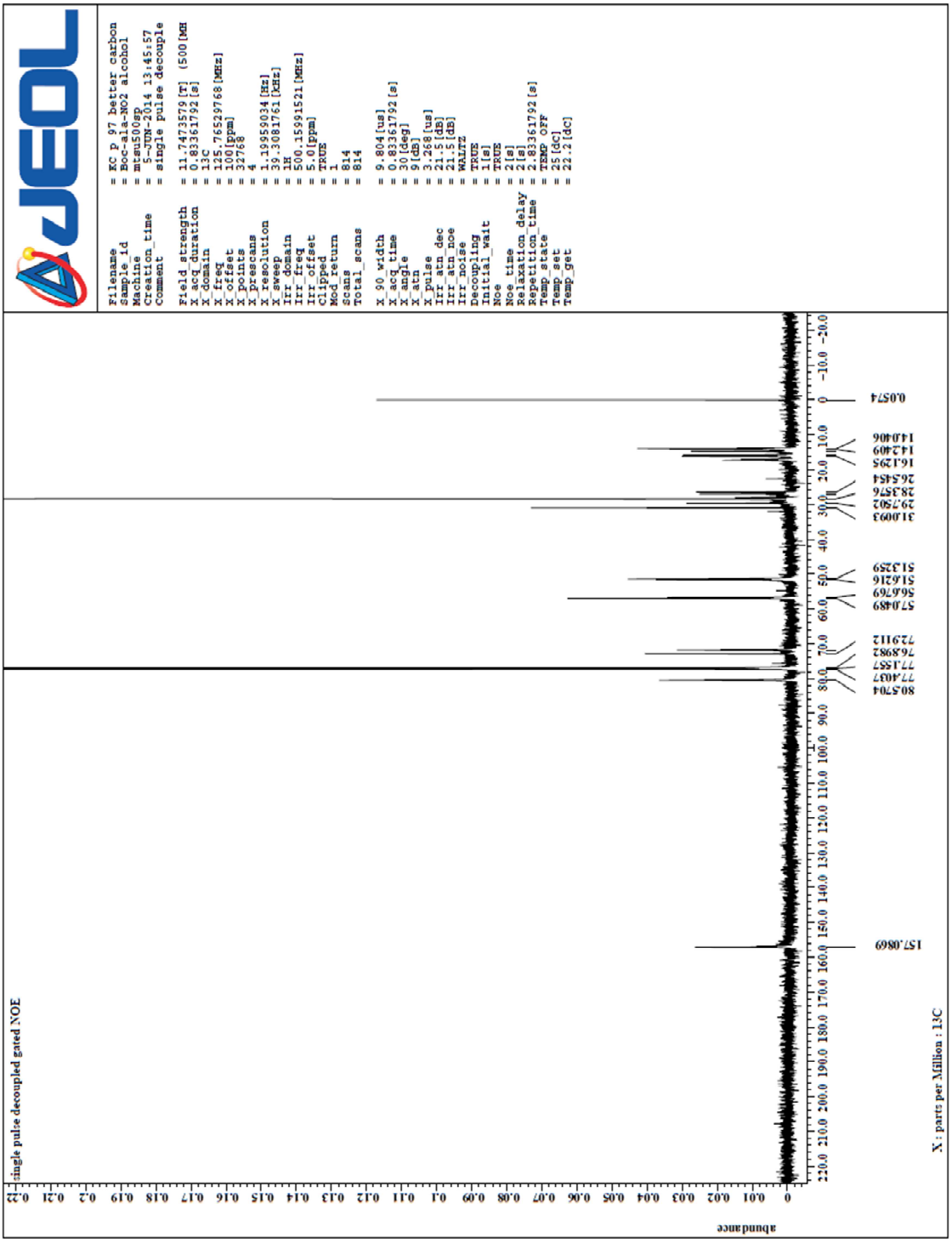


NMR:

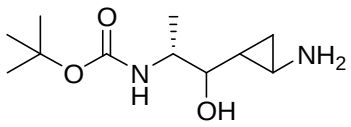
^{1}H-NMR

^{13}C-NMR


Boc-ala-NO2 alcohol
Filename = KC_p_97_better_proton
Sample_id = KC_p_97_better_proton
Machine = mtsu500sp
Creation_time = 5-JUN-2014 11:26:42
Comment = Boc-ala-NO2 alcohol
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 32
Total_scans = 32
X_90_width = 15.534[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.767[us]
Irr_mode = Off
Tri_mode = Off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.6[dC]
abundance
X : parts per Million : 1H
7.2537
4.8717
4.8557
4.8282
4.2614
4.2533
4.2465
3.8285
3.7517
3.6613
3.4666
2.1405
2.0248
2.0099
1.7809
1.7694
1.4179
1.4041
1.1911
1.1774
1.1636
-0.0377
1.17
1
1.79
2.16
1.12
11.36
4.61


JEOL
Filename = EC_p_97_better_carbon
Sample_id = Boc-ala-NO2 alcohol
Machine = mteu500sg
Creation_time = 5-JUN-2014 13:45:57
Comment = single pulse decouple
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76529768[MHz]
X_offset = 100[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Clipped = TRUE
Mod_return = 1
Scans = 814
Total_scans = 814
X_90_width = 9.804[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.268[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 22.2[dC]
single pulse decoupled gated NOE
abundance
X : parts per Million : 13C
157.0869
80.5704
77.4037
77.1557
76.8982
72.9112
57.0489
56.6769
51.6216
51.3259
31.0093
29.7502
28.3576
26.5454
16.1295
14.2409
14.0406
0.0574

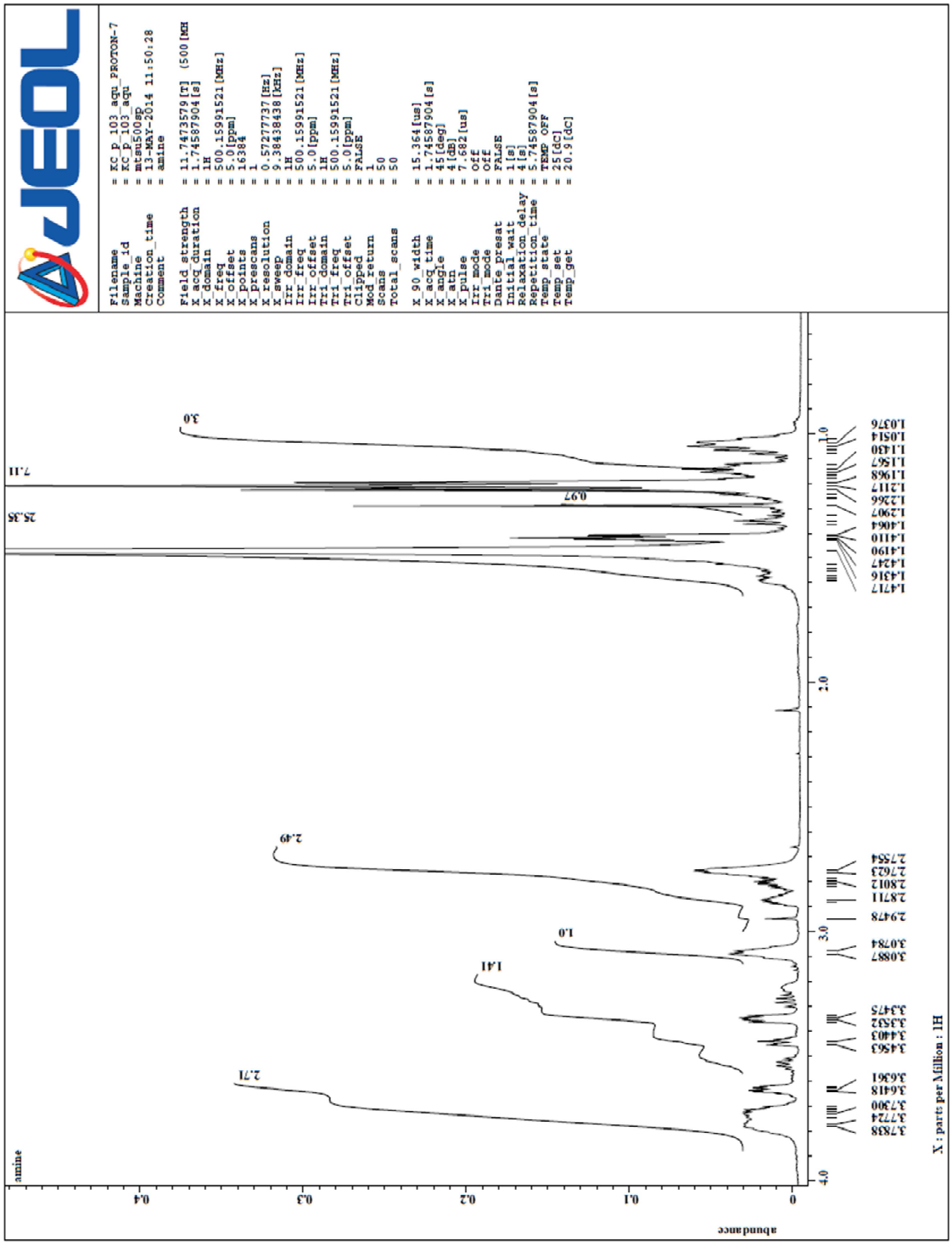
***tert*-Butyl N-[(1R)-4-amino-2-hydroxy-1-methyl-hexyl]carbamate (24)**

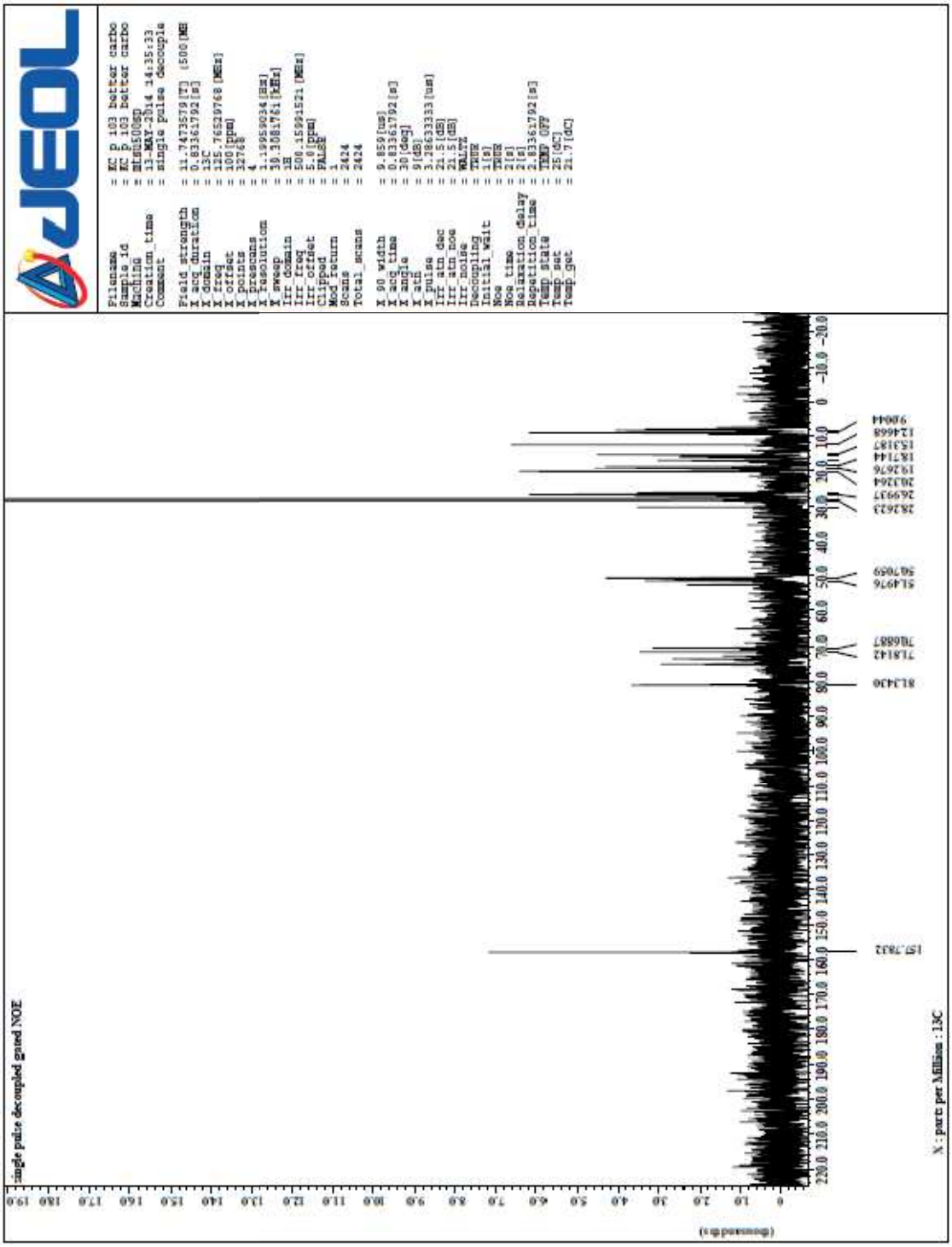


NMR:

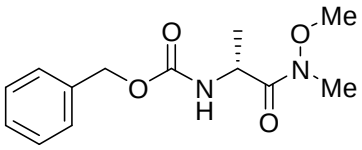
^{1}H-NMR

^{13}C-NMR


JEOL
Filename = EC_p_103_aqu_PROTON-7
Sample_id = EC_p_103_aqu
Machine = mtsu500gp
Creation_time = 13-MAY-2014 11:50:28
Comment = amine
Field_strength = 11.7473579[T] (500[MH
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 1
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521[MHz]
Irr_offset = 5.0[ppm]
Tri_domain = 1H
Tri_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 50
Total_scans = 50
X_90_width = 15.364[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.682[us]
Irr_mode = off
Tri_mode = off
Dante_presat = FALSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 20.9[dC]
amine
abundance
X : parts per Million : 1H
2.71
1.41
1.0
2.49
0.97
25.35
7.11
3.0

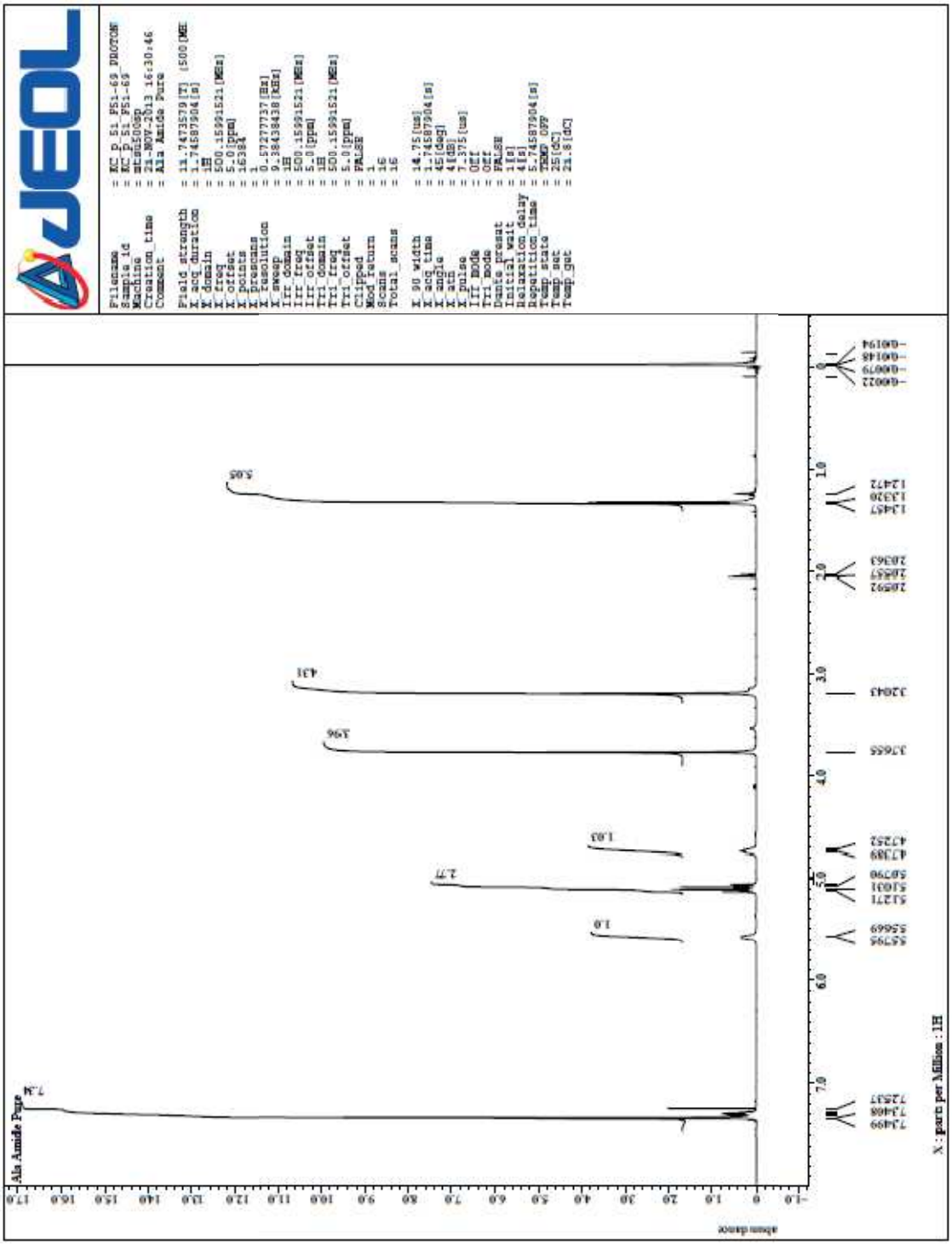

JEOL
Filename = EC_p_103_better_carbo
Sample_id = EC_p_103_better_carbo
Machine = ECA500@pc
Creation_time = 13-MAY-2014_14:35:33
Comment = single_pulse_decouple
Field_strength = 11.74735759[T] (500[MH
X_acq_duration = 0.83361792[s]
X_domain = 13C
X_freq = 125.76520768 [MHz]
X_offset = 100.[ppm]
X_points = 32768
X_prescans = 4
X_resolution = 1.19959034[Hz]
X_sweep = 39.3081761[kHz]
Irr_domain = 1H
Irr_freq = 500.15991521 [MHz]
Irr_offset = 5.0[ppm]
Clipped = FALSE
Mod_return = 1
Scans = 2424
Total_scans = 2424
X_90_width = 9.859[us]
X_acq_time = 0.83361792[s]
X_angle = 30[deg]
X_atn = 9[dB]
X_pulse = 3.28633333[us]
Irr_atn_dec = 21.5[dB]
Irr_atn_noe = 21.5[dB]
Irr_noise = WALTZ
Decoupling = TRUE
Initial_wait = 1[s]
Noe = TRUE
Noe_time = 2[s]
Relaxation_delay = 2[s]
Repetition_time = 2.83361792[s]
Temp_data = TEMP OFF
Temp_set = 25[dC]
Temp_get = 21.7[dC]
single pulse decoupled gated NOE
X : parts per Million : 13C
(Normalized)
157.7832
81.3430
77.1142
76.6887
51.4976
50.7059
28.2623
26.9937
20.3264
19.2676
18.7144
15.3187
12.4668
9.2044

(R)-benzyl 1-(methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate (26)

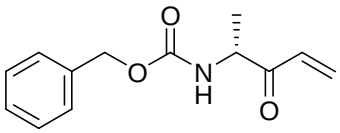


NMR:

^{1}HNMR

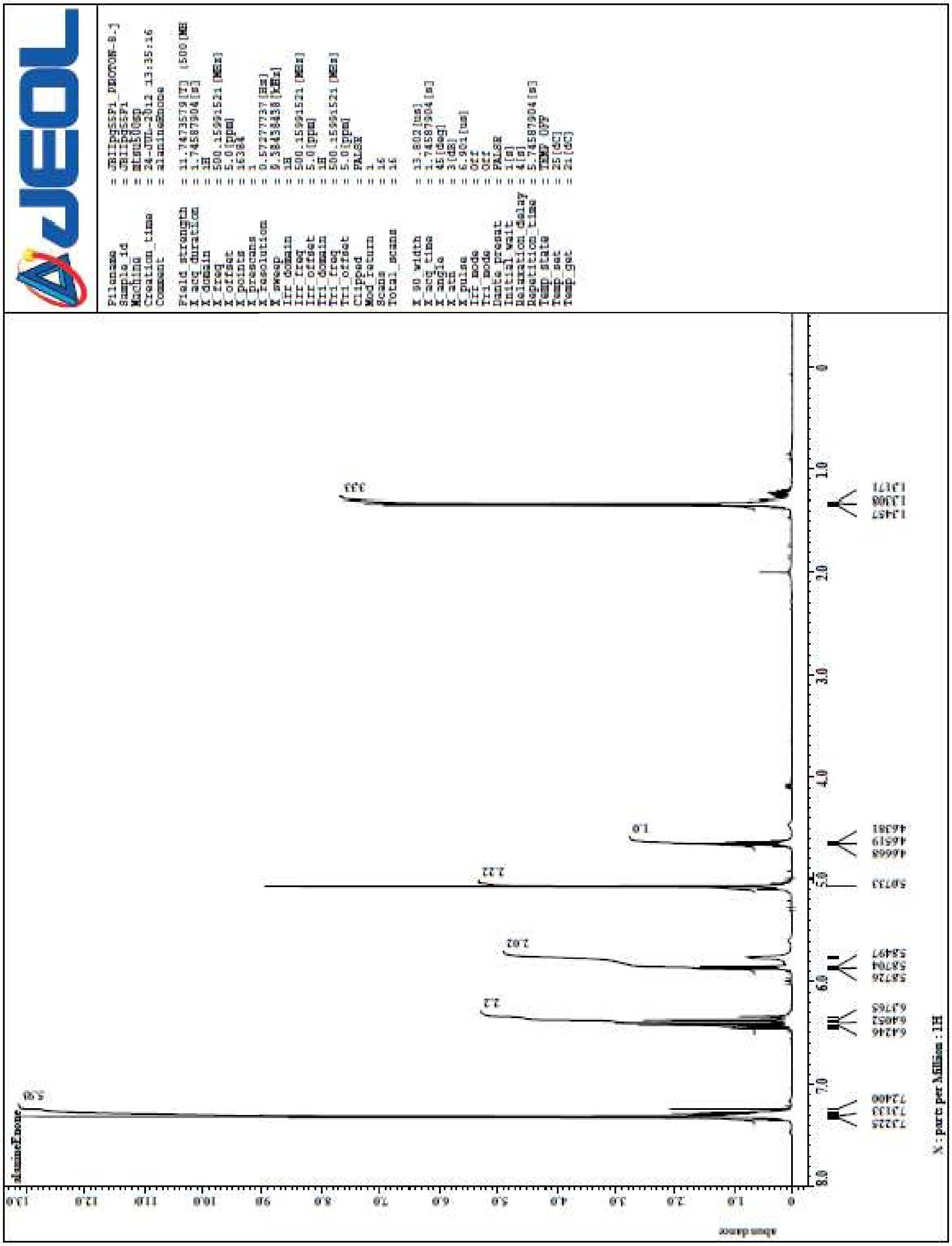

JEOL
Filename = KC_p_51_P51-69_PROTON
Sample_id = KC_p_51_P51-69
Machine = mem500dp
Creation_time = 23-NOV-2013 16:30:46
Comment = Ala Amide Pure
Field_strength = 11.7473579[T] (500[MHz
X_acq_duration = 1.74587904[s]
X_domain = 1H
X_freq = 500.15991521[MHz]
X_offset = 5.0[ppm]
X_points = 16384
X_prescans = 0
X_resolution = 0.57277737[Hz]
X_sweep = 9.38438438[kHz]
I1r_domain = 1H
I1r_freq = 500.15991521[MHz]
I1r_offset = 5.0[ppm]
I1i_domain = 1H
I1i_freq = 500.15991521[MHz]
Tri_offset = 5.0[ppm]
Clipped = PULSE
ModFeturn = 1
Scans = 16
Total_scans = 16
X_90_width = 14.75[us]
X_acq_time = 1.74587904[s]
X_angle = 45[deg]
X_atn = 4[dB]
X_pulse = 7.375[us]
I1r_mode = OFF
Tri_mode = OFF
Dante_presat = PULSE
Initial_wait = 1[s]
Relaxation_delay = 4[s]
Repetition_time = 5.74587904[s]
Temp_state = TEMP OFF
Temp_set = 25[dC]
Temp_get = 25.8[dC]
Ala Amide Page
7.34
5.05
4.31
3.96
1.03
2.77
1.0
ppm
X : parts per Million : 1H

(R)-benzyl 3-oxopent-4-en-2-ylcarbamate (27)

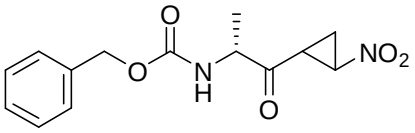


NMR:

^{1}H-NMR

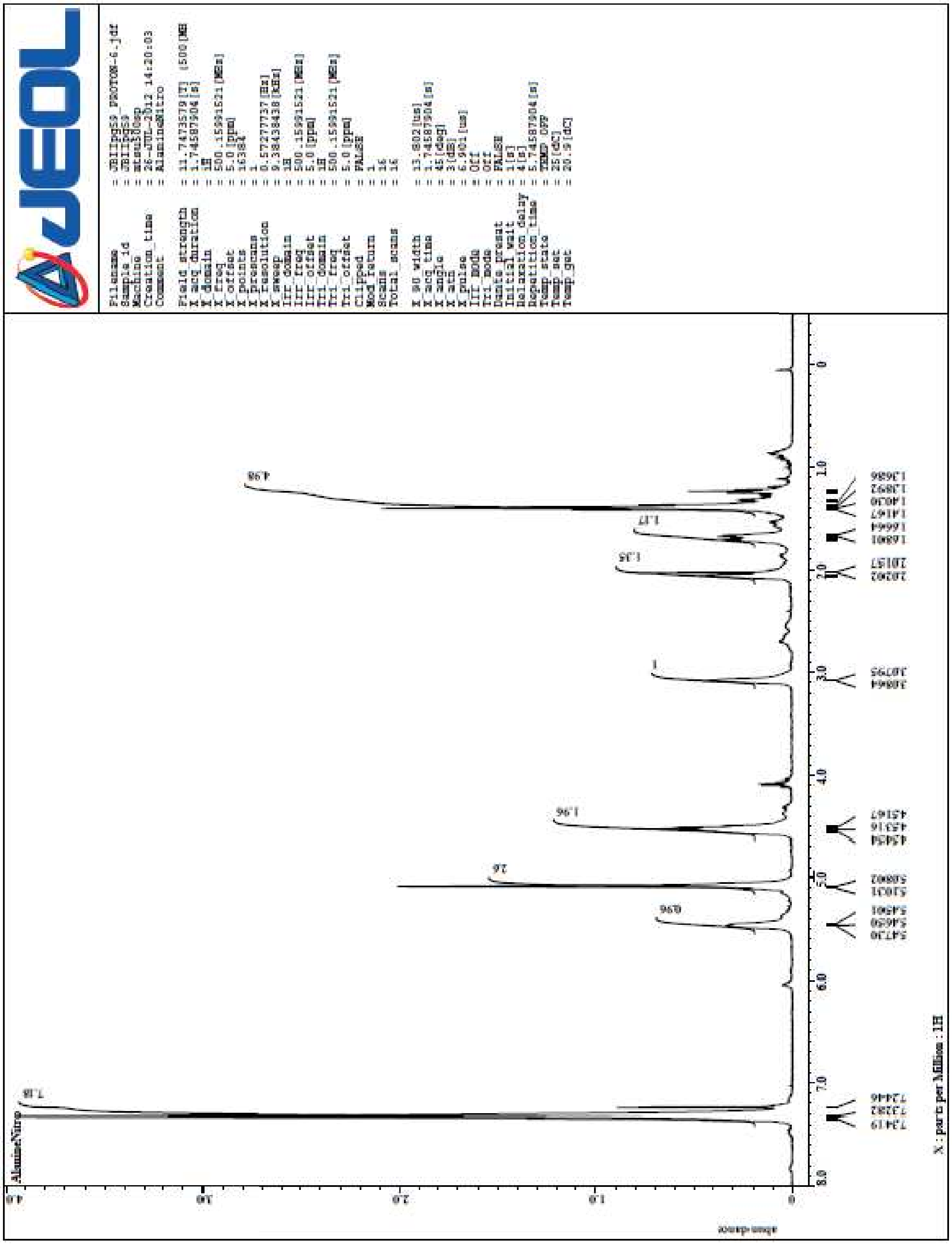


Benzyl (R)-1-(2-nitrocyclopropyl)-1-oxopropan-2-ylcarbamate (28)



NMR:

^{1}H-NMR


AlanineNitro
X : parts per Million : 1H